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J. K. RICHARDSON

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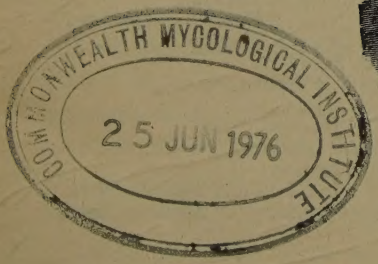
STUDIES ON THE COCCACEAE

III. THE NITROGEN METABOLISM OF THE MICROCOCCI

G. J. HUCKER



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TECHNICAL BULLETIN No. 101

STUDIES ON THE COCCACEAE

III. THE NITROGEN METABOLISM OF THE MICROCOCCI.

G. J. HUCKER

SUMMARY

A study of twelve representative strains of micrococci indicates that the nitrogen requirements are very similar for all members of this group of cocci. All of the cultures investigated had the power to utilize peptone and complex polypeptide fractions of commercial peptones, while various non-dializable fractions were not available in the absence of fermentable carbohydrates.

Many of the cultures had the power to produce biuret-giving substances in the medium, when grown with ammonium phosphate and biuret-free digested proteins as the only source of nitrogen.

The presence of the suspended organisms in the medium under test did not appear to affect the results.

INTRODUCTION

In a study of any group of organisms it is necessary to ascertain their food requirements before their relationship to each other or to other related groups can be determined satisfactorily. The carbon metabolism of bacteria has been studied in great detail in many cases, and fine distinctions have been drawn in applying the results to an arrangement of the organisms into groups. Such conclusions have been based largely upon the ability of the organisms under investigation to produce demonstrable amounts of acid when certain carbohydrates are furnished as sources of carbon. Certain groups appear to have a particular affinity for breaking down certain carbohydrate molecules, while others are unable to utilize these sources of carbon as a means of securing energy. Altho so-called fermentation studies assist in separating certain groups, the results appear to be too variable to warrant their universal acceptance as fundamental characters useful for the purposes of classification. It must be admitted, how-

During the course of the investigation helpful and timely criticisms have been made by Dr. L. F. Rettger of Yale University and Dr R. S. Breed of this Station.

ever, that certain groups of organisms, such as members of the colon-typhoid group, possess the ability constantly to attack certain carbohydrates to the exclusion of others, and in such instances a study of the availability of various carbon sources becomes important.

As the usual nitrogen sources provided in ordinary media may provide both carbon and nitrogen, and as the nature of the nitrogen required appears to throw considerable light upon the general characteristics of the organism, it appears that a knowledge of the available forms in which the organisms in question utilize this chief source of growth is of great value. It is not only the type of nitrogen required that is important, but also a knowledge of the by-products formed. These by-products may indicate the chemical processes which take place within the cell.

It is the object of this part of the investigation dealing with the micrococci to determine the form in which this group requires its nitrogen and the nature of the by-products produced when certain nitrogenous products are provided as food. In making such a study, the possible fundamental relationships of various members of the group should be kept in mind, a point which is important because of the fact that many systematists at present divide this closely related group into two genera. If a division of the group into saprophytes (micrococci) and parasites (staphylococci) is warranted, it would seem natural to expect to find some recognizable differences in the nitrogen metabolism of the two groups.

A knowledge of the limitations of the micrococci as to nitrogen requirements and of their action upon various nitrogenous substances may also throw light on the activity of the group in relation to various industrial and agricultural processes with which certain members of the group are closely associated, *viz.*, spoilage of various foods, ripening of cheese, etc.

PREVIOUS STUDIES

The nitrogen metabolism of many different types of bacteria has been studied under a multitude of conditions. The object of these investigations has been definitely to ascertain (a) the ultimate form in which nitrogen is taken into the cell, (b) the form in which this nitrogen is most available when furnished in a certain medium, and (c) the action of various organisms on various types of nitrogenous compounds.

It is generally agreed that bacteria ultimately absorb their nitrogen (other than the nitrifying bacteria) either as NH_2 or NH_3, but the form in which the NH_2 or NH_3 is furnished may affect considerably its availability. Some saprophytic types can utilize a digested protein containing only amino acids, while a large number prefer NH_2 when in the form of polypeptids, and a small number of strictly parasitic organisms prefer their NH_2 in the form of complex linkages from animal proteins, such as blood serum, etc., which have undergone little or no hydrolysis.

Bainbridge (1911)[1] demonstrated that purified and unaltered animal proteins would not produce growth when supplied to aerobic and faculative anaerobic bacteria as the only source of nitrogen. He believed that this observation applied only to the aerobes, however, and that the anaerobes had the power to attack native proteins. Moreover, Bainbridge found that *Bacillus typhosus*, *B. coli communis*, *B. pyocyaneus*, and *Staphylococcus pyogenes albus* do not appreciably break down pure or unchanged egg-albumen or serum protein, even in the presence of sufficient non-protein nitrogenous food to insure vigorous bacterial growth.

In 1912, Rettger and Sperry showed that even anaerobes could not use native proteins (egg-white) when all other sources of nitrogen were excluded; and in 1915, they offered additional data in substantiation of this point. In the latter investigation similar results were secured when several vegetable proteins were tried. However, the anaerobes could utilize native protein if small amounts of peptone or other decomposition products were added to initiate growth.

In 1916, Rettger, Berman, and Sturgis showed that coagulated egg-albumen was resistant to bacterial action and, in addition, that proteoses and peptones were not attacked by non-gelatin-liquifying types of organisms. The group of gelatin liquefiers, however, readily attacked the proteoses and peptones.

Berman and Rettger (1916 and 1918) confirmed the observation that non-liquefying types could not attack the proteose fraction of Witte's peptone, and showed further that the non-liquefiers and some liquefiers are feeble in their action upon unfractionated peptone. Furthermore, the ability of an organism to liquefy gelatin is not a sure indication of its proteolytic properties. It was also pointed out that

[1]Refers to Bibliography, page 44.

not only native proteins but also purified proteoses are resistant to direct bacterial attack.

Benton in 1919 studied the action of *B. proteus*, *B. pyocyaneus*, staphylococcus, and *B. typhosus* on ascitic fluid, broth, and mixtures of both. She found that on ascitic fluid all of these organisms markedly diminished the amount of coagulable protein in the first 36 hours, while in broth only *B. proteus* and *B. pyocyaneus* were proteolytic. In this investigation, however, the protein of the ascitic fluid was not chemically purified and no doubt traces of decomposition products may have been present.

Altho considerable work has been done regarding the type of organic nitrogenous material available for various bacteria, it was also thought probable that different bacteria may be characterized by their selection of specific amino-acid or other simple compounds, when offered in pure state. If such characteristics are found, nitrogen assimilation can be used in the same way as carbohydrate fermentation for routine diagnostic tests.

As early as 1899 Jordan used a synthetic medium in which various simple nitrogenous substances were offered as sources of nitrogen in studying fluorescence production by bacteria.

Galimard and Lacomme (1907) used 24 different species in studying the effect of various amino acids upon bacterial growth. It was found that 13 of the 24 developed when arginine was present, while phenylalanine failed to support growth in any instance.

Dalimier and Lancereaux (1913) prepared a biuret-free digested product called "opsine" which presumably contained all the amino acids necessary and grew many of the more exacting pathogens. However, certain types failed to develop indicating that altho NH_2 or NH_3 is needed eventually as a source of nitrogen, it must be furnished in a more complex linkage or nearer the natural protein state.

In 1914, Franzen and Egger found that glycocoll, alanine, and asparagine would support the growth of *B. prodigiosus*.

Gordon (1917) used nitrogenous substances of known composition and found that *B. coli*, *B. paratyphosus*, *B. pyocyaneus*, and *B. proteus* could grow when several different amino acids and ammonium salts were furnished as the only sources of nitrogen, while several species of staphylococci and streptococci and *B. typhosus* would not grow under such conditions.

Koser and Rettger (1919) showed that chemically pure amino-acids seem to form a rather broad group in so far as their ability to

furnish nitrogen to the organisms in question is concerned. Some organisms were found to reproduce thru several generations in solutions of various amino acids, while urea, taurine, creatine, etc., appeared to be even inferior to amino acids as sources of nitrogen. Staphylococci as well as several other closely related types would not develop in a synthetic medium containing combinations of amino acids as the only source of nitrogen.

More recently Braun and Cahn-Bronner (1921) have shown that staphylococci will not utilize ammonia nitrogen when furnished as the only source of nitrogen.

In studying the effect of bacteria upon proteins, the majority of the earlier workers used sugar-free media, thus compelling the organisms to obtain their carbon for energy from the protein molecule. Glenn in 1911, however, pointed out that in a medium containing both sugar and protein the carbohydrate is first attacked and secondly the protein, providing the acidity developed is not too great. In the same year (1911) Kendall reported that "fermentation takes precedence over putrefaction;" and in 1912 Kendall and Farmer reported more in detail regarding the action of sugars upon nitrogen metabolism and concluded that sugars have a "protein-sparing" action in bacterial as well as animal metabolism.

That carbohydrates also check the production of proteolytic enzymes was shown by Kendall and Walker (1915) by the fact that *B. proteus* failed to produce such enzymes in carbohydrate-containing media, while in sugar-free media proteolytic ferments could be easily demonstrated.

In 1918, Berman and Rettger corroborated Kendall's findings that carbohydrates have a protein-sparing action, while many workers, Baginski (1889), Péré (1892), Distaso (1913), De Graff (1909), Rougentzoff (1913), and others, have shown that various carbohydrates will inhibit the production of indol, a by-product of putrefaction. In working with various species of staphylococci, Salvioli (1894) and Rosenthal and Patai (1914) found very little difference between the nitrogen requirements of avirulent and virulent strains, while Emmerling (1897) decided that staphylococci could attack any protein and many fatty acids.

In work dealing with definite chemical substances such as amino acids, coagulable proteins, etc., actual determinations could be made to indicate whether or not a given substance had been utilized and to what degree. However, when it was found that certain decomposition

products must be present to initiate or promote growth, a question arose as to the proper method of determining proteolysis.

Crabill and Reed (1915) noted the digestion and utilization of the substance in question by direct observation of the medium in petri dishes.

For sometime Kendall and his co-workers have used ammonia production as an index of protein utilization, while Rettger and his associates have claimed that NH_3 is not indicative of protein utilization as ammonia may be produced from amino acid and other simple substances. Rettger, Berman, and Sturgis (1916), as well as Peckham (1897), has used the quantitative biuret test as suggested by Vernon (1904), together with the formol titration of Sörensen (1907) which, according to Rice (1915), is more satisfactory than the Van Slyke (1913) method for the determination of free amino acids.

TECHNIC

SELECTION OF CULTURES FOR THE STUDY OF NITROGEN METABOLISM

Twelve strains were selected as follows: *Micrococcus aureus*, *M. aurantiacus*, *M. albus*, *M. epidermidis*, *M. ureae*, *M. casei*, *M. flavus*, *M. conglomeratus*, *M. citreus*, *M. varians*, *M. cinnebareus*, and *M. roseus*.

The origin and derivation of these names and the general characteristics of these species will be taken up in a later part of this report (Technical Bulletin No. 102). As pointed out in this bulletin these cultures represent the more important species of the genus.

In the present work the Van Slyke micro-method of determining free amino acids, the formol titration of amino acids, the quantitative biuret, and the determination of ammonia have been selected as giving an indication of the changes taking place in a nitrogenous substratum when acted upon by bacteria of the genus *Micrococcus*.

It is evident that the results of a single determination at any one stage of the growth is not sufficient to warrant conclusions being drawn; nor can a large amount of information be secured by growing organisms in a given test substance which is analyzed after a prolonged incubation and the results compared with an analysis of the original medium. Any true study of the nitrogen metabolism of a group should be based upon tests made at regular intervals during growth in order that the sequence of events may be plotted. Such a method has been followed in the present investigation as the organisms have been inoculated into test substances and determinations made on the 1st, 3rd, 6th, 9th, 14th, and 30th day of growth.

BIURET TEST

Standards were prepared in a series of Nessler tubes which consisted of 0. 1 cc. to 2 cc. of a 0.1 per cent Witte's peptone in graduations of 0.1 cc. to which had been added, in each tube, 20 cc. of 4 per cent NaOH and 2 cc. of a N/100 $CuSO_4$ solution. To test the unknown, 1 cc. of the substance in question was added to a Nessler tube, together with the same amount of $CuSO_4$ and NaOH as used in the standards and color comparisons made with the standards. The readings were expressed in the terms of the percentage of Witte's peptone in the standard to which the unknown was equal in biuret-giving properties. The results can be converted to mgms. of Witte's peptone by calculating the actual amount of peptone in the standards used (Vernon, 1904).

FORMOL TITRATION

The modification ("Method B") of the original Sörensen (1908) method as proposed by Brown (1923) was used. It differs from the original principally in the fact that the titrations are made on a H-ion concentration scale rather than using phenolphthalein as indicator.

VAN SLYKE METHOD FOR DETERMINATION OF FREE AMINO ACIDS

The Van Slyke method for the determination of amino nitrogen was found extremely valuable if the necessary precautions were followed to secure constant results. In all cases the determinations were repeated until relatively constant results could be obtained which in some instances proved to be difficult. However, it was found that if care was taken in selecting the quality of $NaNO_3$ used and if a constant time (five minutes) for the shaking was employed, the results were fairly uniform.

Difficulty was experienced in securing the proper grade of caprylic alcohol. Several different lots were tried and many appeared to give a large blank reading, while others gave a much smaller check reading even when the other reagents were the same. Butyl alcohol was tried in some cases to prevent foaming and the results appeared to be satisfactory. In those cases where the foaming did not affect the reaction neither the caprylic nor butyl alcohol was used.

The micro-method as described by Van Slyke (1913) was followed as closely as possible, and the calculations of the amount of amino

nitrogen present were determined with due corrections for temperature, etc.

AMMONIA DETERMINATION

Ammonia was determined by the method outlined by Folin (1902) and his associates. A given amount of the medium to be tested was made alkaline with Na_2CO_3, aspirated into a standard acid solution, and the amount of ammonia which was taken up in the acid determined by titration with standard alkali.

This method appeared to be fairly accurate. Controls containing a known amount of ammonia were included with each determination as checks.

TURBIDITY

The amount of growth appearing in the tubes was roughly determined by comparison with the nephelometer standards as suggested by MacFarland (1907), with the exception that the standards were made in broth instead of distilled water. A small amount of thymol was added as a preservative. With such standards the determination of the actual amount of growth in the medium could be determined with a fair degree of accuracy as the barium precipitate in the broth gave a cloudiness very characteristic of a heavy growth of the organisms.

DATA

From the earlier work cited above it appears to be a well-established fact that the micrococci will not utilize native proteins when such are furnished as the only source of nitrogen or even produce any appreciable effect upon such substances if decomposition products are present to initiate and promote growth. It has been questioned whether the proteoses and peptones can be utilized. In order to determine which decomposition product of protein furnishes the most available source of nitrogen, the nitrogen changes which take place when the micrococci are grown under ordinary conditions with commercial peptone (Difco) as the source of nitrogen were studied.

Difco peptone contains products representing all stages of digestion and it was thought that a study of the effect of the micrococci upon such a nitrogen source would give an indication as to the possible form in which the nitrogen is preferred by this group and also throw some light upon the general problem of nitrogen metabolism of bacteria.

ACTION OF THE MICROCOCCI UPON DIFCO PEPTONE

Eleven species of the micrococci listed above were inoculated into a medium consisting of

Difco peptone.....5 grams
H_2O (distilled).....1.0 liter
Reaction adjusted to pH 7.0

and a duplicate series into a similar medium to which 1 per cent of chemically pure anhydrous glucose was added. The inoculated tubes were incubated at 30° C. and tubes withdrawn at 1-, 3-, 6-, 14-, and 30-day intervals and tested by the methods mentioned above.

The first apparent result (Table 1) is the similarity of the curves as plotted when the different species of micrococci were allowed to act upon Difco peptone. Little difference in the nitrogen changes can be noted between *M. aureus*, which is probably the most highly specialized parasite, and *M. ureae* which is probably a true saprophyte. Such an observation indicates that the large group of micrococci are fundamentally similar in their nitrogen metabolism. These results do not give any indication that the group should be divided into two genera, the one including the parasitic and the other the saprophytic forms.

That the organisms have only a slight effect on the biuret-giving portion of the peptone, which constitutes the more complex forms of nitrogen present, is indicated by the very slight drop in the biuret, both in the carbohydrate-free medium and in the glucose medium. It is evident that under such conditions the micrococci do not preferably attack the more complex protein linkages for their nitrogen.

Upon examination of the results as obtained by the Van Slyke and formol titration methods, striking differences can be noted between the sugar-free and carbohydrate media. When the organisms are grown in the absence of glucose, the free amino nitrogen rapidly increases, while in the presence of glucose the amount of the free amino nitrogen in the medium gradually decreases. This is indicated by both the Van Slyke and formol titration methods for determining the amino (also some ammonia) form of nitrogen. These results indicate clearly that in this instance the glucose has a "protein-sparing" action. In the carbohydrate-free medium the organisms were forced to break down the protein linkages to secure carbon and in breaking such linkages more amino groups were made accessible to the action of the nitrous acid (Van Slyke) and more COOH groups were liberated in the methylation of the NH_2 groups by the formalin.[2]

[2]As the nitrous acid used in the Van Slyke method splits off the free NH_2 group, while the formol titration determines the number of COOH groups present, the two readings do not coincide due to the fact that the amount of di-carboxylic and di-amino acids present may vary.

TABLE 1.—THE ACTION OF THE MICROCOCCI UPON DIFCO PEPTONE.

No. of culture	Name	Time of incubation	Without dextrose					With dextrose				
			Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration				Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*			*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
385	*M. aureus*	1	0.2517	0.140	0.000	1.00	1.40	1.180	0.490	0.035	1.00	1.00
385	*M. aureus*	3	0.2530	0.140	0.000	3.00	1.40	0.360	0.420	0.038	1.00	1.00
385	*M. aureus*	6	0.3320	0.240	0.070	4.00	1.40	0.820	0.560	0.064	1.00	1.00
385	*M. aureus*	9	0.5878	0.320	0.064	3.00	1.30	1.020	0.420	0.070	1.00	1.00
385	*M. aureus*	14	0.7896	0.520	0.070	4.50	1.30	0.700	0.350	0.070	3.00	0.90
385	*M. aureus*	30	0.9822	0.320	0.070	5.00	1.30	0.274	0.350	0.070	2.50	0.90
	Uninoculated	—	0.5134	0.140	—	—	1.40	1.180	0.630	—	—	1.00
238	*M. albus*	1	0.2517	0.160	0.000	1.00	1.40	0.830	0.630	0.000	0.50	1.00
238	*M. albus*	3	0.2538	0.160	0.014	3.00	1.40	0.690	0.490	0.000	0.50	1.00
238	*M. albus*	6	0.5091	0.240	0.014	4.00	1.40	1.030	0.490	0.000	1.00	1.00
238	*M. albus*	9	1.0630	0.580	0.000	3.00	1.30	0.300	0.490	0.049	1.50	1.00
238	*M. albus*	14	1.1562	0.280	0.014	4.50	1.30	0.452	0.490	0.070	3.00	1.00
238	*M. albus*	30	1.0990	0.850	0.028	4.50	1.30	0.279	0.490	0.070	2.00	0.90
	Uninoculated	—	0.5134	0.140	—	—	1.40	1.180	0.630	—		1.00
196	*M. flavus*	1	0.2797	0.190	0.014	1.00	1.40	0.830	0.070	0.315	0.50	1.00
196	*M. flavus*	3	0.2820	0.190	0.000	3.00	1.40	1.330	0.490	0.350	0.50	0.85
196	*M. flavus*	6	0.7212	0.320	0.028	4.00	1.40	0.820	0.630	0.070	1.00	1.00
196	*M. flavus*	9	1.1469	0.850	0.028	3.00	1.40	0.820	0.700	0.000	2.00	0.90
196	*M. flavus*	14	1.4100	0.850	0.028	4.50	1.30	0.848	0.490	0.000	3.00	0.90
196	*M. flavus*	30	1.3346	0.820	0.021	4.50	1.30	0.387	0.420	0.000	2.50	0.90
	Uninoculated	—	0.5134	0.140	—	—	1.40	1.180	0.630	—	—	1.00
349	*M. cinnebareus*	1	0.2797	0.160	0.000	1.00	1.40	0.320	0.630	0.070	0.25	1.00
349	*M. cinnebareus*	3	0.3384	0.190	0.000	3.00	1.40	0.750	0.350	0.070	0.25	1.00

349	*M. cinnebareus*	6	0.8602	0.520	0.000	4.00	1.40	1.030	0.560	0.070	1.00	1.00
349	*M. cinnebareus*	9	1.4538	0.560	0.064	4.00	1.30	0.730	0.430	0.070	3.00	1.00
349	*M. cinnebareus*	14	0.8178	0.320	0.042	4.50	1.30	0.450	0.420	0.035	3.00	1.00
349	*M. cinnebareus*	30	0.8879	0.700	0.133	4.50	1.30	0.380	0.350	0.070	3.00	0.90
	Uninoculated	—	0.5134	0.140	—	—	1.40	1.180	0.630	—	—	1.00
62	*M. conglomeratus*	1	0.2517	0.140	0.028	1.00	1.40	1.450	0.560	0.035	1.00	1.00
62	*M. conglomeratus*	3	0.3384	0.190	0.028	3.00	1.40	0.840	0.490	0.042	1.00	1.00
62	*M. conglomeratus*	6	0.2775	0.240	0.028	4.00	1.40	1.100	0.700	0.070	1.50	1.00
62	*M. conglomeratus*	9	0.6153	0.480	0.042	4.00	1.40	0.820	0.420	0.070	2.00	1.00
62	*M. conglomeratus*	14	0.7896	0.320	0.000	4.50	1.30	0.700	0.420	0.028	3.00	1.00
62	*M. conglomeratus*	30	1.3760	0.850	0.042	4.50	1.30	0.276	0.350	0.035	3.00	0.90
	Uninoculated	—	0.5134	0.140	—	—	1.40	1.180	0.630	—	—	1.00
88	*M. ureae*	1	0.2797	0.190	0.000	1.00	1.40	0.800	0.630	0.000	0.50	1.00
88	*M. ureae*	3	0.3384	0.190	0.042	3.00	1.40	0.450	0.420	0.000	0.75	1.00
88	*M. ureae*	6	1.3760	0.440	0.000	4.00	1.40	1.030	0.560	0.000	2.00	1.00
88	*M. ureae*	9	0.6432	0.440	0.035	4.00	1.40	0.820	0.420	0.070	2.00	1.00
88	*M. ureae*	14	0.5922	0.350	0.105	4.50	1.30	0.840	0.420	0.070	1.50	0.90
88	*M. ureae*	30	0.8048	0.560	0.014	4.50	1.30	0.887	0.350	0.070	1.50	0.90
	Uninoculated	—	0.5134	0.140	—	—	1.40	1.180	0.630	—	—	1.00
213	*M. roseus*	1	0.3076	0.160	0.000	1.00	1.40	1.190	0.420	0.000	0.50	1.00
213	*M. roseus*	3	0.3102	0.220	0.035	3.00	1.40	0.740	0.350	0.000	0.50	1.00
213	*M. roseus*	6	1.3760	0.440	0.000	4.00	1.40	0.890	0.560	0.000	1.00	1.00
213	*M. roseus*	9	1.1469	0.580	0.028	4.00	1.40	0.820	0.420	0.000	3.00	1.00
213	*M. roseus*	14	1.2972	0.880	0.000	4.50	1.30	0.840	0.420	0.070	1.50	0.90
213	*M. roseus*	30	0.9710	0.580	0.049	4.50	1.30	0.498	0.350	0.070	2.50	0.90
	Uninoculated	—	0.5134	0.140	—	—	1.40	1.180	0.630	—	—	1.00
111	*M. casei*	1	0.2597	0.140	0.000	1.00	1.40	0.1669	0.220	0.000	0.25	1.40
111	*M. casei*	3	0.3666	0.190	0.014	3.00	1.40	0.1665	0.240	0.000	1.00	1.40
111	*M. casei*	6	0.6950	0.240	0.133	3.00	1.40	0.1669	0.220	0.000	3.00	1.40
111	*M. casei*	9	0.6711	0.320	0.014	4.00	1.30	0.1402	0.220	—	4.00	1.40
111	*M. casei*	14	0.9870	0.580	0.000	4.50	1.30	0.1398	0.190	0.021	4.00	1.40
111	*M. casei*	30	1.3760	0.760	0.126	4.50	1.30	0.0930	0.190	0.070	4.00	1.30
	Uninoculated	—	0.5134	0.140	—	—	1.40	0.1947	0.240	—	—	1.40

TABLE 1.—THE ACTION OF THE MICROCOCCI UPON DIFCO PEPTONE.—*Concluded.*

No. of culture	Name	Time of incubation	Without dextrose					With dextrose				
			Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration				Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*			*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
191	*M. epidermidis*	1	0.2597	0.160	—	1.00	1.40	0.1669	0.240	0.000	0.50	1.40
191	*M. epidermidis*	3	0.3384	0.220	0.000	3.00	1.40	0.1387	0.220	0.098	1.00	1.40
191	*M. epidermidis*	6	0.5368	0.240	0.000	4.00	1.40	0.1669	0.190	0.000	3.00	1.40
191	*M. epidermidis*	9	1.0076	0.440	0.028	4.00	1.40	0.1683	0.190	0.070	4.00	1.40
191	*M. epidermidis*	14	1.1562	0.240	0.042	4.50	1.30	0.1398	0.190	0.070	4.00	1.30
191	*M. epidermidis*	30	0.5368	0.520	0.072	4.50	1.30	0.0640	0.160	0.035	4.00	1.30
	Uninoculated	—	0.5134	0.140	—	—	1.40	0.1947	0.240	—	—	1.40
167	*M. citreus*	1	0.2517	0.140	0.000	1.00	1.40	0.1391	0.220	0.028	0.50	1.40
167	*M. citreus*	3	0.2820	0.190	0.028	3.00	1.40	0.1665	0.190	0.126	1.00	1.40
167	*M. citreus*	6	0.6673	0.280	0.000	4.00	1.40	0.1391	0.190	0.126	3.00	1.40
167	*M. citreus*	9	1.1190	0.630	0.000	4.00	1.40	0.1398	0.190	0.140	4.00	1.40
167	*M. citreus*	14	0.9180	0.440	0.021	4.50	1.30	0.1683	0.160	0.140	4.00	1.40
167	*M. citreus*	30	0.8048	0.320	0.000	4.50	1.30	0.0930	0.160	0.126	4.00	1.40
	Uninoculated	—	0.5134	0.140	—	—	1.40	0.1947	0.240	—	—	1.40
80	*M. varians*	1	0.2517	0.160	—	1.00	1.40	0.1947	0.220	0.112	0.50	1.40
80	*M. varians*	3	0.3102	0.220	0.000	3.00	1.40	0.1942	0.220	0.072	1.00	1.40
80	*M. varians*	6	0.7212	0.320	0.000	3.00	1.40	0.1947	0.220	0.070	3.00	1.40
80	*M. varians*	9	1.0910	0.580	0.000	4.00	1.40	0.1963	0.190	0.064	4.00	1.40
80	*M. varians*	14	0.7896	0.480	0.007	4.50	1.20	0.1678	0.140	0.021	4.00	1.40
80	*M. varians*	30	0.7771	0.880	0.021	4.50	1.20	0.1213	0.140	0.014	4.00	1.40
	Uninoculated	—	0.5134	0.140	—	—	1.40	0.1947	0.240	—	—	1.40

In the medium containing the glucose the gradual decrease in the amount of free amino acid is probably due to the fact that the organisms needing only a small amount of nitrogen for their growth, as shown by Rettger, can secure the necessary amount from the loosely attached free amino groups which are gradually utilized as the growth continues. The large amount of carbon needed for growth is, of course, furnished by the glucose.

An interesting observation can be noted in regard to the production of ammonia by the micrococci. Kendall and his co-workers have found this character fairly constant in studying various other groups, but in the present investigation the results were very irregular and unsatisfactory. As can be noted in Table 1 the amounts of ammonia varied from day to day, and it is probable that in many instances ammonia was being utilized and liberated simultaneously. If this was not in equal proportions, it would tend to affect the results. The method of determination was constantly checked with known solutions to guard against faulty technic.

It is evident from these results that glucose has a "protein-sparing" action and that the micrococci prefer the simpler linkages even in the absence of a fermentable carbohydrate as evidenced by the slight decrease in the biuret and the decrease in the free amino acids when glucose was furnished, allowing the organisms to secure the most easily available nitrogen without the need of breaking down the molecule for carbon.

ACTION OF MICROCOCCI UPON FRACTIONS OF PEPTONE

The micrococci produce a vigorous growth upon peptone media, but as the nitrogen thus furnished is in a multitude of forms ranging from the non-dialyzable proteose portion to the simple and amino acids, it is not clear which stage is the most available. However, it is indicated from the results noted above that the more complex linkages are probably untouched.

In order to ascertain the fraction of the peptone most available, the peptone was fractionated into four portions as follows:

a. *Proteose extract.* 350 grams of Difco peptone were dissolved in two liters of water, saturated with $(NH_4)_2 SO_4$, and the precipitate filtered, suspended in water, and dialyzed for eight days. Toluene was added to prevent contamination. The material remaining in the bag was dried and added to the media as a source of nitrogen.

TABLE 2.—THE ACTION OF THE MICROCOCCI UPON THE "COMPLEX PEPTIDE" FRACTION OF DIFCO PEPTONE.

No. of culture	Name	Time of incubation	Without dextrose					With dextrose				
			Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret	Amino-acid nitorgen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration				Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*			*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
385	*M. aureus*	1	0.3937	0.280	0.000	0.25	1.00	0.5316	0.240	0.25	0.25	1.20
385	*M. aureus*	3	0.4451	0.315	0.000	1.00	1.00	0.4914	0.240	0.014	0.50	1.20
385	*M. aureus*	6	0.3329	0.240	0.014	2.00	1.00	0.5211	0.220	0.028	0.75	1.20
385	*M. aureus*	9	0.4192	0.308	0.000	3.00	0.90	0.4416	0.190	0.000	1.00	1.20
385	*M. aureus*	14	1.1531	0.320	0.028	3.00	1.00	0.3567	0.160	0.000	2.00	1.10
385	*M. aureus*	30	1.0769	0.440	0.363	3.50	0.90	0.3567	0.160	0.000	2.00	1.10
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20
238	*M. albus*	1	0.3375	0.280	0.000	0.25	1.00	0.5037	0.280	0.014	0.50	1.20
238	*M. albus*	3	0.3895	0.350	0.070	1.00	1.00	0.4644	0.280	0.000	0.75	1.20
238	*M. albus*	6	0.3606	0.320	0.021	2.00	1.00	0.4389	0.220	0.140	1.00	1.20
238	*M. albus*	9	0.8879	0.601	0.000	3.00	0.80	0.4416	0.190	0.000	2.00	1.20
238	*M. albus*	14	0.9277	0.520	0.063	3.5	1.00	0.3841	0.160	0.000	3.00	1.10
238	*M. albus*	30	1.1906	0.320	0.056	3.5	0.90	0.3841	0.160	0.000	2.00	1.10
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20
196	*M. flavus*	1	0.3937	0.280	0.063	0.25	1.00	0.5874	0.220	0.000	0.50	1.20
196	*M. flavus*	3	0.3895	0.350	0.000	1.0	1.00	0.4368	0.220	0.000	0.75	1.20
196	*M. flavus*	6	0.7771	0.480	0.000	2.0	0.90	0.4115	0.190	0.000	0.50	1.20
196	*M. flavus*	9	0.9433	0.580	0.000	3.0	1.00	0.3864	0.160	0.148	1.00	1.20
196	*M. flavus*	14	0.6187	0.180	0.042	3.5	1.00	0.1150	0.160	0.000	2.00	1.10
196	*M. flavus*	30	0.8319	0.440	0.035	3.5	0.80	0.4115	0.140	0.000	2.00	1.10
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20
349	*M. cinnebareus*	1	0.3375	0.350	0.070	0.25	1.00	0.6432	0.220	0.000	0.50	1.20
349	*M. cinnebareus*	3	0.3895	0.280	0.063	1.00	1.00	0.4095	0.220	0.000	0.75	1.20

349	*M. cinnebareus*	6	0.2775	0.280	0.000	2.00	1.00	0.3841	0.220	0.000	1.00	1.20
349	*M. cinnebareus*	9	0.9981	0.240	0.105	3.00	0.80	0.5244	0.190	0.000	1.00	1.20
349	*M. cinnebareus*	14	0.8715	0.730	0.063	3.50	0.80	0.4389	0.140	1.700	1.00	1.10
349	*M. cinnebareus*	30	0.5953	0.410	0.000	3.50	0.80	0.3019	0.140	0.328	1.00	1.10
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20
62	*M. conglomeratus*	1	0.3937	0.295	0.049	0.25	1.00	0.6990	0.220	0.000	0.50	1.20
62	*M. conglomeratus*	3	0.3339	0.350	0.056	1.00	1.00	0.5460	0.220	0.070	1.00	1.20
62	*M. conglomeratus*	6	0.2578	0.440	0.000	2.00	1.00	0.3841	0.220	0.000	1.00	1.20
62	*M. conglomeratus*	9	0.3329	0.320	0.028	3.00	1.00	0.3864	0.160	0.070	2.00	1.20
62	*M. conglomeratus*	14	0.6748	0.610	0.014	3.50	—	0.3841	0.140	0.000	2.00	1.10
62	*M. conglomeratus*	30	1.1906	0.520	0.000	3.50	0.60	0.2745	0.140	0.000	2.00	1.10
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20
88	*M. ureae*	1	0.3937	0.280	0.000	0.25	1.00	0.5316	0.280	0.070	0.50	1.20
88	*M. ureae*	3	0.3895	0.280	0.000	1.00	1.00	0.5187	0.190	0.028	0.75	1.20
88	*M. ureae*	6	0.9987	0.520	0.007	2.00	0.90	0.4115	0.190	0.028	1.00	1.20
88	*M. ureae*	9	1.3760	0.410	0.070	3.00	1.00	0.4140	0.190	0.000	2.00	1.20
88	*M. ureae*	14	0.5344	0.580	0.028	3.50	0.90	0.2841	0.160	0.014	2.00	1.10
88	*M. ureae*	30	1.1623	0.610	0.000	3.50	0.90	0.2571	0.160	0.070	1.50	1.10
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20
213	*M. roseus*	1	0.3375	0.350	0.000	0.25	1.00	0.5037	0.240	0.000	0.50	1.20
213	*M. roseus*	3	0.3895	0.350	0.000	1.00	1.00	0.4368	0.220	0.000	0.75	1.20
213	*M. roseus*	6	0.2578	0.240	0.000	2.00	1.00	0.4389	0.220	0.070	2.00	1.20
213	*M. roseus*	9	1.4187	0.440	0.035	3.00	1.00	0.4416	0.160	0.140	3.00	1.20
213	*M. roseus*	14	0.8715	0.520	0.000	3.50	0.80	0.3567	0.140	0.000	4.00	1.10
213	*M. roseus*	30	1.4740	0.690	0.063	3.50	0.80	0.3019	0.160	0.000	4.00	1.10
	Uninoculated	—	0.3916	0.280	—	—	—	0.6293	0.280	—	—	1.20
111	*M. casei*	1	0.3375	0.350	0.035	0.25	1.00	0.7050	0.240	0.000	0.50	1.20
111	*M. casei*	3	0.3895	0.350	0.007	1.00	1.00	0.5550	0.240	0.063	0.75	1.20
111	*M. casei*	6	0.5091	0.240	0.000	1.00	1.00	0.4173	0.240	0.000	1.00	1.20
111	*M. casei*	9	0.9433	0.850	0.056	3.00	0.80	0.7293	0.190	0.105	2.00	1.20
111	*M. casei*	14	1.7149	0.610	0.000	3.00	0.80	0.4140	0.190	0.000	3.00	1.00
111	*M. casei*	30	0.9071	0.520	0.077	3.50	0.80	0.2517	0.220	0.000	—	1.00
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20

TABLE 2—THE ACTION OF THE MICROCOCCI UPON THE "COMPLEX PEPTIDE" FRACTION OF DIFCO PEPTONE.—*Concluded.*

No. of culture	Name	Time of incubation	Without dextrose					With dextrose				
			Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration				Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*			*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
191	*M. epidermidis*	1	0.3375	0.280	0.070	0.25	1.00	0.5922	0.280	0.056	0.50	1.20
191	*M. epidermidis*	3	0.3339	0.350	0.000	1.00	1.00	0.4414	0.280	0.021	0.75	1.20
191	*M. epidermidis*	6	0.6396	0.440	0.000	2.00	0.90	0.4729	0.240	0.000	1.00	1.20
191	*M. epidermidis*	9	0.5832	0.240	0.000	2.00	1.00	0.8133	0.220	0.000	2.50	1.00
191	*M. epidermidis*	14	1.1250	0.630	0.021	3.50	1.00	0.5244	0.220	0.118	3.00	1.00
191	*M. epidermidis*	30	0.7370	1.125	0.084	3.50	0.80	0.2797	0.240	0.056	—	1.00
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20
167	*M. citreus*	1	0.2812	0.280	0.000	0.25	1.00	0.5922	0.308	0.049	0.50	1.20
167	*M. citreus*	3	0.3339	0.350	0.000	1.00	1.00	0.5368	0.280	0.014	0.75	1.20
167	*M. citreus*	6	1.3760	0.850	0.000	2.00	0.90	0.3895	0.220	0.014	1.00	1.20
167	*M. citreus*	9	0.8879	0.320	0.000	2.00	1.00	0.7853	0.190	0.000	2.00	1.20
167	*M. citreus*	14	0.8434	0.580	0.000	3.50	1.00	0.3864	0.190	0.091	3.00	1.00
167	*M. citreus*	30	0.9071	0.610	0.063	—	0.80	0.3355	0.190	0.063	—	1.20
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20
80	*M. varians*	1	0.2375	0.280	0.000	0.25	1.00	0.6204	0.240	0.070	0.50	1.20
80	*M. varians*	3	0.3895	0.280	0.007	1.00	1.00	0.5091	0.240	0.000	0.75	1.20
80	*M. varians*	6	0.9986	0.820	0.021	2.00	0.90	0.5285	0.240	0.007	1.00	1.00
80	*M. varians*	9	0.5368	0.308	0.000	2.00	0.80	0.7573	0.220	0.021	2.00	1.00
80	*M. varians*	14	0.8996	0.880	0.112	3.50	0.80	0.4140	0.160	0.028	3.00	1.00
80	*M. varians*	30	1.3040	0.580	0.000	—	0.80	0.3076	0.160	0.070	—	1.00
	Uninoculated	—	0.3916	0.280	—	—	1.00	0.6293	0.280	—	—	1.20

TABLE 3.—THE ACTION OF THE MICROCOCCI UPON THE "PROTEOSE EXTRACT" OF DIFCO PEPTONE PLUS DEXTROSE.

No. of culture	Name	Time of incubation	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
385	*M. aureus*.....	1	0.5811	0.308	0.000	0.50	1.00
385	*M. aureus*.....	3	0.6546	0.280	0.063	1.00	1.00
385	*M. aureus*.....	6	0.6050	0.280	0.056	3.00	1.00
385	*M. aureus*.....	9	0.5756	0.220	0.000	3.50	1.00
385	*M. aureus*.....	14	0.4166	0.220	0.007	3.50	0.90
385	*M. aureus*.....	30	0.5020	0.085	0.000	3.00	0.90
	Uninoculated.	—	0.8025	0.320	—	—	1.20
238	*M. albus*.....	1	0.8128	0.280	0.000	0.50	1.20
238	*M. albus*.....	3	0.9276	0.224	0.000	1.00	1.20
238	*M. albus*.....	6	0.5504	0.308	0.000	3.00	1.20
238	*M. albus*.....	9	0.4151	0.220	0.000	3.50	1.20
238	*M. albus*.....	14	0.3068	0.140	0.035	3.50	1.00
238	*M. albus*.....	30	0.2760	0.110	0.098	3.00	1.00
	Uninoculated.	—	0.8025	0.320	—	—	1.20
196	*M. flavus*.....	1	0.7023	0.320	0.000	0.50	1.20
196	*M. flavus*.....	3	0.8184	0.220	0.070	1.00	1.20
196	*M. flavus*.....	6	0.4954	0.240	0.000	3.00	1.20
196	*M. flavus*.....	9	0.5221	0.190	0.014	3.00	1.00
196	*M. flavus*.....	14	0.5813	0.160	0.140	3.50	1.00
196	*M. flavus*.....	30	0.4416	0.140	0.140	3.00	1.00
	Uninoculated.	—	0.8025	0.320	—	—	1.20
349	*M. cinnebareus*.	1	0.6470	0.308	0.000	0.50	1.20
349	*M. cinnebareus*.	3	0.7638	0.280	0.056	1.00	1.20
349	*M. cinnebareus*.	6	0.6604	0.240	0.007	3.00	1.20
349	*M. cinnebareus*.	9	0.4686	0.160	0.007	3.00	1.00
349	*M. cinnebareus*.	14	0.5264	0.190	0.049	3.50	1.00
349	*M. cinnebareus*.	30	0.3864	0.160	0.021	3.00	1.00
	Uninoculated.	—	0.8025	0.320	—	—	1.20
62	*M. citreus*.....	1	0.7023	0.320	0.000	0.50	1.20
62	*M. citreus*.....	3	0.8184	0.308	0.000	1.00	1.20
62	*M. citreus*.....	6	0.4954	—	—	3.00	1.20
62	*M. citreus*.....	9	0.4686	0.240	0.021	3.00	0.80
62	*M. citreus*.....	14	0.3617	0.160	0.140	4.00	0.60
62	*M. citreus*.....	30	—	0.190	0.140	3.00	0.70
	Uninoculated.	—	0.8205	0.320	—	—	1.20
88	*M. ureae*.....	1	0.8128	0.308	0.000	0.25	1.20
88	*M. ureae*.....	3	0.8730	0.320	0.000	0.75	1.20
88	*M. ureae*.....	6	0.5504	0.240	0.007	2.00	1.20
88	*M. ureae*.....	9	0.5756	0.240	0.035	4.00	1.20
88	*M. ureae*.....	14	—	0.140	0.070	4.00	1.20
88	*M. ureae*.....	30	0.5520	0.220	0.160	4.00	1.00
	Uninoculated.	—	0.8205	0.320	—	—	1.20
213	*M. roseus*.....	1	0.5811	0.350	0.000	0.25	1.20
213	*M. roseus*.....	3	0.5460	0.240	0.014	0.75	1.20
213	*M. roseus*.....	6	0.6054	0.220	0.000	2.00	1.20

TABLE 3.—THE ACTION OF THE MICROCOCCI UPON THE "PROTEOSE EXTRACT" OF DIFCO PEPTONE PLUS DEXTROSE.—*Concluded.*

No. of culture	Name	Time of incubation	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
213	*M. roseus*.....	9	0.6291	0.220	0.000	4.00	—
213	*M. roseus*.....	14	0.4166	0.110	0.112	4.00	1.00
213	*M. roseus*.....	30	0.4960	0.140	0.077	4.00	1.00
	Uninoculated..	—	0.8025	0.320	—	—	1.20
111	*M. casei*.....	1	0.6832	0.308	0.063	0.25	1.20
111	*M. casei*.....	3	0.6105	0.280	0.000	1.00	1.20
111	*M. casei*.....	6	0.1130	0.308	0.000	1.00	1.20
111	*M. casei*.....	9	0.6451	0.190	0.063	1.00	1.00
111	*M. casei*.....	14	0.4717	0.160	0.035	2.00	1.00
111	*M. casei*.....	30	0.5087	0.160	0.000	3.00	1.00
	Uninoculated.	—	0.9088	0.320	—	—	1.20
191	*M. epidermidis.*	1	0.8520	0.280	0.056	0.25	1.20
191	*M. epidermidis.*	3	0.5555	0.220	0.028	1.00	1.20
191	*M. epidermidis.*	6	0.1130	0.308	0.000	1.00	1.20
191	*M. epidermidis.*	9	0.7573	0.160	0.056	1.00	1.00
191	*M. epidermidis.*	14	0.8089	0.240	0.028	2.00	1.00
191	*M. epidermidis*	30	0.4522	0.140	0.063	3.00	1.00
	Uninoculated.	—	0.9088	0.320	—	—	1.20
167	*M. citreus*.....	1	0.6830	0.320	0.000	0.25	1.20
167	*M. citreus*.....	3	0.7492	0.240	0.035	1.00	1.20
167	*M. citreus*.....	6	0.5565	0.280	0.042	1.00	1.00
167	*M. citreus*.....	9	0.5329	0.140	0.000	2.00	1.00
167	*M. citreus*.....	14	0.4717	0.220	0.056	2.00	1.00
167	*M. citreus*.....	30	0.3927	0.160	0.126	3.00	1.00
	Uninoculated.	—	0.9088	0.320	—	—	1.20
80	*M. varians*....	1	0.7390	0.308	0.000	0.25	1.20
80	*M. varians*....	3	0.5555	0.280	0.000	1.00	1.20
80	*M. varians*....	6	0.1130	0.240	0.014	1.00	1.00
80	*M. varians*....	9	0.4768	0.240	0.000	1.00	1.00
80	*M. varians*....	14	0.3593	0.190	0.098	2.00	1.00
80	*M. varians*....	30	0.3392	0.140	0.042	3.00	1.00
	Uninoculated.	—	0.9088	0.320	—	—	1.20

b. *Complex peptide extract.* 100 grams of Difco peptone dissolved in water were concentrated to a thick syrup, extracted with 50 per cent alcohol, and filtered. Alcohol was then added to the extract to make the final dilution 80 per cent. The precipitate was filtered, washed with 80 per cent alcohol, dried, and added as a powder to the test medium.

c. *Simple peptide extract.* 100 grams of Difco peptone were dissolved in water, concentrated to a thick syrup, and alcohol added to make the final concentration 50 per cent. The precipitate was filtered, washed with 50 per cent alcohol, and dried.

d. *Amino acid extract.* 100 grams of Difco peptone were extracted, with frequent shakings for two days, with 80 per cent alcohol. The extract was concentrated and powdered.

Altho not sharply defined chemically, these extracts probably represent four stages of different complexity of the protein molecule as found in commercial peptone. The amino-acid extract was practically biuret-free, while the proteose extract gave the deep purple biuret characteristic of that group of compounds. The peptide extracts also gave tests for such compounds, while the complex peptide extract showed a pinkish biuret, indicating that this portion probably contained a large part of the peptones. The results when the various polypeptides were used (Tables 2 and 4) were very similar to those obtained when the entire peptone was used. The amount of growth appeared to be the same, while the growth as evidenced in the change in biuret and amino-acid readings resembled very closely the similar readings when Difco peptone was furnished instead of the polypetide fraction. The glucose appeared to have the same effect upon the nitrogen curves and in no apparent respect did the offering of the polypeptide groups alone as the only source of nitrogen seem to alter the normal growth of the organisms.

In the amino-acid extract (Table 5) the organisms produced a heavy growth with a gradual decrease in the amount of the amino acids present and the formation of a biuret-giving substance. The control which was uninoculated showed a faint biuret reaction, but after 24 to 36 hours growth the inoculated tubes showed a pronounced purple color.

The results as obtained from using the proteose extract (Table 3) were interesting, due to the fact that repeated trials with this non-dialyzable portion of peptone, when furnished as the only source of nitrogen in the absence of glucose, gave no growth, whereas when

TABLE 4.—THE ACTION OF THE MICROCOCCI UPON "SIMPLE POLYPEPTIDE" EXTRACT OF DIFCO PEPTONE.—*Concluded.*

No. of culture	Name	Time of incubation	Without dextrose					With dextrose				
			Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration				Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*			*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
191	*M. epidermidis*	1	0.5034	0.280	0.063	0.25	1.20	0.7050	0.160	0.000	0.50	1.80
191	*M. epidermidis*	3	0.7347	0.320	0.098	0.50	1.20	0.6396	0.110	0.000	2.00	1.80
191	*M. epidermidis*	6	0.8695	0.410	0.000	1.00	1.20	0.5285	0.110	0.021	2.50	1.80
191	*M. epidermidis*	9	1.1623	0.880	0.021	1.00	1.00	0.5328	0.140	0.000	3.00	1.80
191	*M. epidermidis*	14	1.2259	0.700	0.014	2.00	1.00	0.4498	0.140	0.014	3.00	1.80
191	*M. epidermidis*	30	0.8113	0.520	0.091	2.50	0.90	0.3109	0.110	0.063	3.00	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.7332	0.120	—	—	1.80
167	*M. citreus*	1	0.3355	0.308	0.000	0.25	1.20	0.6204	0.140	0.070	0.50	1.80
167	*M. citreus*	3	0.6501	0.280	0.000	0.50	1.00	0.5832	0.140	0.063	2.00	1.80
167	*M. citreus*	6	0.8135	0.380	0.056	1.00	1.00	0.5565	0.110	0.014	2.50	1.80
167	*M. citreus*	9	1.4174	0.320	0.000	1.00	1.00	0.5328	0.110	0.070	3.00	1.80
167	*M. citreus*	14	1.3608	0.630	0.077	2.00	1.00	0.5063	0.085	0.028	3.00	1.80
167	*M. citreus*	30	1.1469	0.440	0.063	2.00	1.00	0.3391	0.110	0.007	3.50	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.7332	0.120	—	—	1.80
80	*M. varians*	1	0.3355	0.320	0.000	0.25	1.20	0.5922	0.140	0.000	0.50	1.80
80	*M. varians*	3	0.6783	0.308	0.042	0.50	1.20	0.5550	0.110	0.105	2.00	1.80
80	*M. varians*	6	0.7855	0.350	0.000	1.00	1.20	0.5285	0.140	0.070	2.50	1.80
80	*M. varians*	9	0.8913	0.280	0.035	1.00	1.00	0.5048	0.140	0.063	3.00	1.80
80	*M. varians*	14	1.0353	0.480	0.000	2.00	1.00	0.5344	0.110	0.070	3.00	1.80
80	*M. varians*	30	1.3980	0.460	0.000	3.00	1.00	0.5372	0.110	0.049	3.00	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.7332	0.140	—	—	1.80

349	*M. cinnebareus*	6	0.8695	0.440	0.182	1.00	1.20	0.5344	0.190	0.014	3.00	2.00
349	*M. cinnebareus*	9	0.8036	0.440	0.042	1.00	1.00	0.5037	0.140	0.112	4.00	1.80
349	*M. cinnebareus*	14	0.8673	0.850	0.098	2.00	0.90	0.4937	0.160	0.119	3.00	1.80
349	*M. cinnebareus*	30	0.7287	0.960	0.000	1.50	0.90	0.3293	0.160	0.140	2.00	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.6483	0.320	—	—	2.00
62	*M. citreus*	1	0.4471	0.240	0.000	0.25	1.20	0.6204	0.280	0.000	0.50	1.80
62	*M. citreus*	3	0.7764	0.640	0.000	0.50	1.20	0.5874	0.240	0.000	1.00	1.80
62	*M. citreus*	6	0.8695	0.440	0.182	1.00	1.20	0.5625	0.220	0.126	2.00	1.80
62	*M. citreus*	9	0.9071	0.410	0.070	1.00	1.00	0.5316	0.160	0.000	4.00	1.80
62	*M. citreus*	14	1.1190	0.850	0.056	1.00	1.00	0.3841	0.160	0.000	4.00	1.80
62	*M. citreus*	30	1.0066	0.580	0.070	2.00	1.00	0.3019	0.160	0.518	4.00	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.6483	0.320	—	—	2.00
88	*M. ureae*	1	0.4192	0.280	0.000	0.25	1.20	0.6483	0.240	0.000	0.50	2.00
88	*M. ureae*	3	0.7764	0.640	0.000	0.50	1.20	0.5874	0.220	0.056	1.00	2.00
88	*M. ureae*	6	0.8975	0.460	0.098	1.00	1.00	0.5344	0.190	0.000	3.00	2.00
88	*M. ureae*	9	0.9071	0.410	0.070	1.00	1.00	0.5037	0.160	0.000	4.00	1.80
88	*M. ureae*	14	1.1190	0.850	0.056	1.00	1.00	0.3567	0.140	0.007	4.00	1.80
88	*M. ureae*	30	1.4574	0.640	0.000	2.50	0.80	0.2745	0.140	0.000	3.00	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.6483	0.230	—	—	2.00
213	*M. roseus*	1	0.3913	0.280	0.105	0.25	1.20	0.5640	0.220	0.000	0.50	1.80
213	*M. roseus*	3	0.8046	0.240	0.063	0.50	1.00	0.5316	0.190	0.000	1.00	1.80
213	*M. roseus*	6	0.5329	0.580	0.070	1.00	1.00	0.5063	0.190	0.343	3.00	1.80
213	*M. roseus*	9	1.1623	0.308	0.056	1.00	1.00	0.5037	0.140	0.210	4.00	1.80
213	*M. roseus*	14	0.5595	0.580	0.063	2.00	1.00	0.4115	0.160	0.000	4.00	1.80
213	*M. roseus*	30	0.6711	0.880	0.000	2.00	1.00	0.3019	0.160	0.000	4.00	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.6483	0.320	—	—	2.00
111	*M. casei*	1	0.3355	0.240	0.000	0.25	1.20	0.7332	0.140	0.063	0.50	1.80
111	*M. casei*	3	0.6501	0.350	0.070	0.50	1.00	0.6950	0.140	0.119	2.00	1.85
111	*M. casei*	6	0.8975	0.440	0.056	1.00	1.00	0.5007	0.140	0.191	0.25	1.80
111	*M. casei*	9	0.9354	0.308	0.126	1.00	1.00	0.5048	0.110	0.056	3.00	1.80
111	*M. casei*	14	0.7543	0.640	0.007	2.00	1.00	0.5063	0.085	0.004	3.00	1.80
111	*M. casei*	30	1.0066	1.020	0.105	2.00	1.00	0.3391	0.085	0.063	3.50	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.7332	0.120	—	—	1.80

TABLE 4.—THE ACTION OF THE MICROCOCCI UPON "SIMPLE POLYPEPTIDE" EXTRACT OF DIFCO PEPTONE.

No. of culture	Name	Time of incubation	Without dextrose					With dextrose				
			Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration				Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*			*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
385	*M. aureus*	1	0.3355	0.220	0.000	0.25	1.00	0.5922	0.250	0.000	0.50	2.00
385	*M. aureus*	3	0.5937	0.308	0.000	0.50	1.20	0.5595	0.240	0.000	1.00	2.00
385	*M. aureus*	6	0.8133	0.320	0.021	0.50	1.20	0.5341	0.220	0.098	2.00	2.00
385	*M. aureus*	9	1.4457	0.580	0.063	1.00	1.00	0.5316	0.220	0.000	2.00	1.80
385	*M. aureus*	14	0.8952	0.308	0.070	2.00	0.90	0.4663	0.160	0.000	4.00	1.80
385	*M. aureus*	30	1.1469	0.610	0.063	2.00	0.90	0.4663	06.10	0.000	3.00	1.80
	Unincoculated	—	0.6190	0.220	—	—	1.20	0.6483	0.320	—	—	2.00
238	*M. albus*	1	0.3076	0.190	0.000	0.25	1.20	0.6204	0.308	0.000	0.50	2.00
238	*M. albus*	3	0.6219	0.320	0.000	0.50	1.20	0.5874	0.240	0.000	1.00	2.00
238	*M. albus*	6	0.8975	0.350	0.000	1.00	1.20	0.4782	0.190	0.042	3.00	2.00
238	*M. albus*	9	0.8036	0.520	0.000	1.00	0.90	0.5037	0.190	0.000	3.00	1.80
238	*M. albus*	14	1.1469	0.320	0.000	2.00	0.90	0.4663	0.140	0.000	3.00	1.80
238	*M. albus*	30	0.8942	0.320	0.070	3.00	0.90	0.3841	0.160	0.000	3.00	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.6483	0.320	—	—	2.00
196	*M. flavus*	1	0.3634	0.240	0.000	0.25	1.20	0.5640	0.320	0.000	0.50	2.00
196	*M. flavus*	3	0.4534	0.308	0.000	0.50	1.20	0.5595	0.220	0.000	1.00	2.00
196	*M. flavus*	6	1.1500	0.580	0.098	1.00	1.20	0.5063	0.140	0.000	3.00	2.00
196	*M. flavus*	9	0.9071	0.820	0.098	1.00	1.00	0.4758	0.160	0.063	4.00	1.80
196	*M. flavus*	14	0.6432	0.580	0.056	2.00	1.00	0.4389	0.140	0.000	3.00	1.80
196	*M. flavus*	30	0.6153	0.520	0.091	2.00	0.90	0.3567	0.140	0.056	3.00	1.80
	Uninoculated	—	0.6190	0.220	—	—	1.20	0.6483	0.320	—	—	2.00
349	*M. cinnebareus*	1	0.3355	0.308	0.070	0.25	1.20	0.5922	0.308	0.042	0.50	2.00
349	*M. cinnebareus*	3	0.6219	0.320	0.000	0.50	1.20	0.5595	0.220	0.000	1.00	2.00

glucose was added as a source of carbon a moderately heavy turbidity could be noted. It is evident that in the absence of an available carbohydrate proteoses are not attacked by the micrococci, but if a source of carbon is added the organisms can secure sufficient nitrogen from the loosely bound amino groups around the complex molecule to initiate and promote growth. The micrococci, however, cannot split the proteose molecule to secure carbon. This observation substantiates the findings of Rettger and his associates that proteoses are not directly available for gelatin non-liquefiers, but also demonstrates the fact that liquefying micrococci cannot attack such substances when furnished in the pure forms. All of the cultures tried, with the exception of *M. roseus*, *M. epidermidis*, and *M. varians*, are gelatin liquefiers.

These observations seem to indicate that the micrococci prefer their nitrogen in the form of polypetids or amino acids and when amino acids are furnished they have the power to produce slight traces of synthetic biuret-giving substances in the medium. However the amino acids, as will be shown later, must be associated with various growth accessory substances.

ACTION OF MICROCOCCI UPON OPSINE

That the micrococci can utilize peptides and amino acids appears certain, and in order to gain further light on the by-products formed when amino acids only were furnished as a source of nitrogen each of the cultures was grown in a medium containing opsine as the only source of nitrogen.

Opsine is a French product first used in this country by Robinson and Rettger (1917) and is a biuret-free product of digestion. It gives no Millon's test[3] but gives a faint precipitate with the silver nitrate test for purines. It gives a slight precipitate upon the addition of phosphotungstic acid and shows a strong xanthoproteic reaction.

The production of ammonia from opsine shows some interesting results (Table 6) as traces only could be found at irregular intervals. Either a constant amount of ammonia was thrown off in the medium and irregularly used or only small amounts were produced at various intervals dependent upon some unknown factor.

Only a slight increase in biuret was noted and this only after long periods of incubation, an observation which differs somewhat from

[3]See Mathews, A. P. Physiological Chemistry. *New York; Wm. Wood, 3 ed. 1921, p. 916.*

TABLE 5.—THE ACTION OF THE MICROCOCCI UPON "AMINO-ACID" EXTRACT OF DIFCO PEPTONE.

No. of culture	Name	Time of incubation	Without dextrose					With dextrose				
			Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration				Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*			*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
385	*M. aureus*	1	0.7380	0.560	0.000	1.00	0.00	1.0122	0.420	0.000	0.50	0.40
385	*M. aureus*	3	0.6785	0.490	0.000	2.00	0.00	0.6785	0.350	0.000	1.50	0.20
385	*M. aureus*	6	0.6187	0.490	0.000	3.00	0.20	0.8436	0.350	0.000	4.00	0.20
385	*M. aureus*	9	0.5670	0.490	0.000	4.00	0.20	0.8953	0.420	0.000	4.00	0.20
385	*M. auresu*	14	0.6190	0.007	4.000	3.50	0.20	0.8335	0.210	0.000	4.00	0.20
385	*M. aureus*	30	0.6785	0.350	0.000	—	0.20	0.8335	0.210	0.000	5.00	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	1.4000	0.350	—	—	0.10
238	*M. albus*	1	0.6785	0.630	0.000	1.00	0.00	1.0122	0.420	0.000	0.50	0.40
238	*M. albus*	3	0.6785	0.420	0.000	2.50	0.000	0.8394	0.420	0.000	2.00	0.20
238	*M. albus*	6	0.6187	0.420	0.000	3.00	0.20	0.8436	0.350	0.000	4.00	0.20
238	*M. albus*	9	0.5670	0.350	0.000	4.00	0.20	0.8953	0.350	0.000	4.00	0.20
238	*M. albus*	14	0.5134	0.350	0.014	4.00	0.00	0.8335	0.210	0.035	4.00	0.20
238	*M. albus*	30	0.6785	0.280	0.028	—	0.20	0.7786	0.210	0.000	5.00	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	1.4000	0.350	—	—	0.10
196	*M. flavus*	1	0.6190	0.490	0.000	1.00	0.00	0.9560	0.420	0.000	0.50	0.40
196	*M. flavus*	3	0.6220	0.560	0.000	2.50	0.00	0.8394	0.350	0.000	2.00	0.20
196	*M. flavus*	6	0.6187	0.420	0.000	3.50	0.20	0.7874	0.350	0.000	3.50	0.20
196	*M. flavus*	9	0.5670	0.350	0.000	4.00	0.20	0.9512	0.280	0.000	5.00	0.20
196	*M. flavus*	14	0.5134	0.350	0.000	4.00	0.00	0.7786	0.210	0.000	5.00	0.20
196	*M. flavus*	30	0.5655	0.280	0.000	—	0.20	0.7380	0.280	0.028	4.50	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	1.4000	0.350	—	—	0.20
349	*M. cinnebareus*	1	0.7939	0.420	0.000	1.00	0.00	1.1246	0.420	0.014	0.50	0.40
349	*M. cinnebareus*	3	0.6785	0.560	0.007	2.50	0.00	0.8953	0.420	0.000	2.00	0.20

349	*M. cinnebareus*	6	0.6750	0.490	0.000	3.00	0.20	0.8436	0.350	0.000	4.00	.020
349	*M. cinnebareus*	9	0.6237	0.350	0.000	4.50	0.20	.9512	0.280	0.035	5.00	0.20
349	*M. cinnebareus*	14	0.5134	0.280	0.000	3.50	0.00	0.8884	0.280	0.028	5.00	0.20
349	*M. cinnebareus*	30	0.5089	0.280	0.000	—	0.20	0.7786	0.210	0.000	4.50	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	1.4000	0.350	—	—	0.20
62	*M. citreus*	1	0.6190	0.350	0.000	1.00	0.00	1.0122	0.420	0.000	0.50	0.40
62	*M. citreus*	3	0.6220	0.420	0.000	2.50	0.00	0.9512	0.350	0.014	2.00	0.20
62	*M. citreus*	6	0.6187	0.350	0.000	3.50	0.20	0.7874	0.420	0.000	4.00	0.20
62	*M. citreus*	9	0.5670	0.350	0.000	4.00	0.20	1.0071	0.350	0.000	5.00	0.20
62	*M. citreus*	14	0.5595	0.280	0.000	4.00	0.00	0.9433	0.210	0.014	5.00	0.20
62	*M. citreus*	30	0.5089	0.280	0.007	—	0.20	0.8884	0.210	0.000	5.00	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	1.4000	0.350	—	—	0.20
88	*M. ureae*	1	0.5134	0.420	1.000	1.00	0.00	1.4000	0.420	0.000	0.50	0.40
88	*M. ureae*	3	0.6220	0.280	0.007	2.50	0.00	1.0071	0.350	0.000	2.00	0.20
88	*M. ureae*	6	0.5061	0.350	0.000	3.00	0.20	0.7874	0.350	0.000	3.50	0.20
88	*M. ureae*	9	0.4626	0.420	0.000	4.00	0.20	0.8953	0.350	0.000	5.00	0.20
88	*M. ureae*	14	0.5595	0.280	0.000	4.00	0.00	1.0531	0.210	0.007	5.00	0.20
88	*M. ureae*	30	0.4524	0.210	0.000	—	0.20	0.9433	0.280	0.000	5.00	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	1.4000	0.350	—	—	0.10
213	*M. roseus*	1	0.6785	0.490	0.000	1.00	0.40	0.7312	0.420	0.035	0.50	0.40
213	*M. roseus*	3	0.5089	0.350	0.000	2.50	0.00	0.6785	0.350	0.007	2.00	0.20
213	*M. roseus*	6	0.5625	0.280	0.000	2.50	0.20	0.6500	0.420	0.035	4.00	0.20
213	*M. roseus*	9	0.5670	0.350	0.000	3.50	0.20	0.8953	0.280	0.000	5.00	0.20
213	*M. roseus*	14	0.5595	0.350	0.000	4.00	0.00	0.8884	0.420	0.000	5.00	0.20
213	*M. roseus*	30	0.5089	0.420	0.000	—	0.20	0.9982	0.420	0.000	5.00	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	1.4000	0.350	—	—	0.10
111	*M. casei*	1	0.5134	0.490	0.000	1.00	0.00	0.7332	0.350	0.000	1.00	0.00
111	*M. casei*	3	0.5089	0.280	0.000	2.50	0.00	0.4440	0.280	0.000	2.50	0.00
111	*M. casei*	6	0.5061	0.350	0.000	3.50	0.20	0.6677	0.280	0.500	3.00	0.00
111	*M. casei*	9	0.5134	0.350	0.000	4.00	0.00	0.6171	0.280	0.028	4.50	0.20
111	*M. casei*	14	0.5134	0.350	0.000	4.00	0.00	0.5625	0.210	0.000	4.00	0.20
111	*M. casei*	30	0.5655	0.350	0.000	—	0.20	0.5089	0.210	0.028	4.50	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	0.7896	0.350	—	—	0.00

TABLE 5—THE ACTION OF THE MICROCOCCI UPON "AMINO-ACID" EXTRACT OF DIFCO PEPTONE.—*Concluded.*

No. of culture	Name	Time of incubation	Without dextrose					With dextrose				
			Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration				Van Slyke	Formol titration			
		Days	*Mgs. per. cc.*	*Mgs. per. cc.*	*Mgs. per cc.*			*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
191	*M. epidermidis*	1	0.6785	0.560	0.007	1.00	0.00	0.6768	0.350	0.000	1.00	0.00
191	*M. epidermidis*	3	0.6220	0.490	0.000	2.50	0.00	0.4995	0.315	0.000	2.50	0.00
191	*M. epidermidis*	6	0.5625	0.350	0.007	3.00	0.20	0.5016	0.350	0.000	4.00	0.20
191	*M. epidermidis*	9	0.5193	0.280	0.028	3.75	0.10	0.5049	0.210	0.014	4.00	0.20
191	*M. epidermidis*	14	0.5595	0.280	0.000	4.00	0.00	0.5061	0.280	0.014	4.00	0.20
191	*M. epidermidis*	30	0.5089	0.420	0.000	—	0.20	0.4524	0.210	0.000	4.50	0.20
	Uninoculated	—	0.7939	0.630	—	—	2.10	0.7896	0.350	—	—	0.00
167	*M. citreus*	1	0.6190	0.490	0.000	1.00	0.00	0.6204	0.280	0.000	1.00	0.00
167	*M. citreus*	3	0.5625	0.490	0.000	2.50	0.00	0.6660	0.350	0.004	3.00	0.00
167	*M. citreus*	6	0.6187	0.350	0.000	3.00	0.20	0.4451	0.420	0.028	4.00	0.00
167	*M. citreus*	9	0.6237	0.420	0.000	4.00	0.10	0.4488	0.210	0.021	4.00	0.20
167	*M. citreus*	14	0.5595	0.280	0.000	4.00	0.00	0.4499	0.210	0.000	5.00	0.20
167	*M. citreus*	30	0.5089	0.490	0.000	—	0.20	0.4524	0.140	0.014	5.00	0.20
	Uninoculated	—	0.7939	0.630	—	—	1.10	0.7896	0.350	—	—	0.00
80	*M. varians*	1	0.5595	0.490	0.000	1.00	0.00	0.7332	0.280	0.000	1.00	0.00
80	*M. varians*	3	0.5625	0.490	0.000	2.50	0.20	0.6660	0.280	0.000	3.00	0.00
80	*M. varians*	6	0.5625	0.350	0.000	3.00	0.20	0.5565	0.280	0.000	4.00	0.00
80	*M. varians*	9	0.6237	0.420	0.000	4.00	0.10	0.5610	0.280	0.000	4.00	0.20
80	*M. varians*	14	0.5134	0.350	0.000	4.00	0.00	0.5061	0.210	0.000	4.00	0.20
80	*M. varians*	30	0.5655	0.420	0.042	—	0.20	0.4524	0.280	0.000	5.00	0.20
	Uninoculated	—	0.7939	0.630	—	—	0.10	0.7896	0.350	—	—	0.20

the results found when the amino acid extract of Difco peptone was used. In this latter instance, a biuret reaction was discernable within 24 hours to two days. A heavier growth was also found in the case of opsine than when the amino extract Difco peptone was used, no doubt due to the fact that a different complex of amino acid was present.

The amino-acid curve showed an expected gradual decrease with a corresponding curve evidenced by the results of the formol titration.

EFFECT OF THE PRESENCE OF SUSPENDED ORGANISMS UPON THE RESULTS

Many investigators have felt that the method of growing organisms in a liquid medium and removing an aliquot portion of the medium containing the organisms will not give a true indication as to what has taken place. It has been suggested that the organisms suspended in the portion being tested may affect the results, due to the fact that various amounts of nitrogenous substances may be bound up in the cell. As the changes in the substratum alone are desired, it may be possible that substances may be present in the suspended cells in sufficient amounts to affect the various tests quantitatively.

In order to determine if the presence of the organisms had any effect upon the results, the cultures under observation were grown in Difco peptone and in the biuret-free opsine broth, both sugar-free. At various intervals tubes were withdrawn and centrifuged, the bacteria-free fluid removed and tested as "media" (Tables 7 and 8), and the organisms re-suspended in saline to the same concentration as the original mixture of broth and organisms. This saline suspension was tested and recorded as "organisms" in the tabular results.

It can be seen that the organisms in the same concentration of suspension as found normally growing in the medium gave no biuret reaction even after 30 days incubation and no increase in amino nitrogen.[4] The biuret reading of the medium when the organisms were grown in a biuret-giving substratum (Table 7) was faintly decreased, while the amino acid reading increased. This observation checks with the previous results obtained when the organisms and medium were tested together.

In the cultures grown in the biuret-free medium (Table 8) the biuret appeared in the medium only and not in the suspension of the organisms. That this biuret-giving substance is the result of autolysis of

[4]The amounts of amino-nitrogen noted in the tables correspond to the amount of nitrogen gas obtained when only the reageants were used in the Van Slyke apparatus.

TABLE 6.—THE ACTION OF THE MICROCOCCI UPON OPSINE.

No. of culture	Name	Time of incubation	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
385	*M. aureus*.....	1	0.6190	0.630	0.000	1.00	0.00
385	*M. aureus*.....	3	0.6187	0.630	0.000	3.00	0.00
384	*M. aureus*.....	6	0.5625	0.560	0.000	4.00	0.00
385	*M. aureus*.....	9	0.5193	0.560	0.000	5.00	0.00
385	*M. aureus*.....	14	0.5134	0.490	0.000	5.00	0.00
385	*M. aureus*.....	30	0.5134	0.280	0.000	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00
238	*M. albus*.....	1	0.5595	0.560	0.000	1.00	0.00
238	*M. albus*.....	3	0.5625	0.560	0.000	3.00	0.00
238	*M. albus*.....	6	0.5625	0.560	0.028	4.00	0.00
238	*M. albus*.....	9	0.5193	0.560	0.000	5.00	0.00
238	*M. albus*.....	14	0.5134	0.490	0.000	5.00	0.00
238	*Ml albus*.....	30	0.4575	0.350	0.007	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00
196	*M. flavus*.....	1	0.5595	0.630	0.000	2.00	0.00
196	*M. flavus*.....	3	0.5625	0.560	0.028	3.50	0.00
196	*M. flavus*.....	6	0.5625	0.560	0.000	4.50	0.00
196	*M. flavus*.....	9	0.5193	0.560	0.000	5.00	0.00
196	*M. flavus*.....	14	0.4575	0.350	0.007	5.00	0.00
196	*M. flavus*.....	30	0.3916	0.420	0.028	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00
349	*M. cinnebareus.*	1	0.6785	0.630	0.000	2.00	0.00
349	*M. cinnebareus.*	3	0.5625	0.560	0.000	3.50	0.00
349	*M. cinnebareus.*	6	0.5625	0.490	0.000	5.00	0.00
349	*M. cinnebareus.*	9	0.5690	0.490	0.000	5.00	0.00
349	*M. cinnebareus.*	14	0.5134	0.420	0.014	5.00	0.00
349	*M. cinnebareus.*	30	0.3916	0.280	0.000	5.00	0.20
	Uninoculated.	—					
62	*M. congl'm'r's.*	1	0.6190	0.560	0.000	2.00	0.00
62	*M. congl'm'r's.*	3	0.6187	0.490	0.000	3.50	0.00
62	*M. congl'm'r's.*	6	0.5061	0.630	0.000	4.00	0.00
62	*M. congl'm'r's.*	9	0.5670	0.490	0.014	5.00	0.00
62	*M. congl'm'r's.*	14	0.5595	0.490	0.028	5.00	0.00
62	*M. congl'm'r's.*	30	0.4575	0.210	0.000	5.00	0.20
	Uninoculated.	—					
88	*M. ureae*.....	1	0.5595	0.630	0.000	2.00	0.00
88	*M. ureae*.....	3	0.5625	0.560	0.000	3.50	0.00
88	*M. ureae*.....	6	0.5625	0.360	0.000	5.00	0.00
88	*M. ureae*.....	9	0.5670	0.420	0.000	5.00	0.00
88	*M. ureae*.....	14	0.5595	0.420	0.000	5.00	0.00
88	*M. ureae*.....	30	0.5134	0.420	0.014	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00
213	*M. roseus*.....	1	0.5595	0.630	0.000	2.00	0.00
213	*M. roseus*.....	3	0.5625	0.630	0.000	3.50	0.00

TABLE 6—THE ACTION OF THE MICROCOCCI UPON OPSINE.—*Concluded.*

No. of culture	Name	Time of incubation	Amino-acid nitrogen		Ammonia nitrogen	Turbidity	Biuret
			Van Slyke	Formol titration			
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
213	*M. roseus*.....	6	0.5061	0.490	0.028	5.00	0.00
213	*M. roseus*.....	9	0.5670	0.490	0.014	5.00	0.00
213	*M. roseus*.....	14	0.5134	0.490	0.000	5.00	0.00
213	*M. roseus*.....	30	0.5134	0.490	0.000	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00
111	*M. casei*.....	1	0.6190	0.560	0.000	2.00	0.00
111	*M. casei*.....	3	0.6157	0.490	0.000	3.50	0.00
111	*M. casei*.....	6	0.5625	0.490	0.000	5.00	0.00
111	*M. casei*.....	9	0.5670	0.560	0.000	5.00	0.00
111	*M. casei*.....	14	0.5134	0.630	0.000	5.00	0.00
111	*M. casei*.....	30	0.3916	0.280	0.000	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00
191	*M. epidermidis.*	1	0.6785	0.630	0.000	2.00	0.00
191	*M. epidermidis.*	3	0.6750	0.560	0.000	3.50	0.00
191	*M. epidermidis.*	6	0.5625	0.560	0.000	5.00	0.00
181	*M. epidermidis.*	9	0.5670	0.490	0.014	5.00	0.00
191	*M. epidermidis.*	14	0.5595	—	0.000	5.00	0.00
191	*M. epidermidis.*	30	0.4575	0.350	0.000	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00
167	*M. citreus*.....	1	0.5595	0.630	0.000	2.00	0.00
167	*M. citreus*.....	3	0.5625	0.490	0.000	3.50	0.00
167	*M. citreus*.....	6	0.5625	0.490	0.000	5.00	0.00
167	*M. citreus*.....	9	0.5193	0.490	0.000	5.00	0.00
167	*M. citreus*.....	14	0.5134	0.630	0.007	5.00	0.00
167	*M. citreus*.....	30	0.4575	0.280	5.00	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00
80	*M. varians*.....	1	0.5595	0.630	0.000	2.00	0.00
80	*M. varians*.....	3	0.5625	0.560	0.000	3.50	0.00
80	*M. varians*.....	6	0.5061	0.560	0.000	5.00	0.00
80	*M. varians*.....	9	0.5193	0.560	0.007	5.00	0.00
80	*M. varians*.....	14	0.5134	—	0.000	5.00	0.00
80	*M. varians*.....	30	0.3916	0.350	0.000	5.00	0.20
	Uninoculated.	—	0.6190	0.630	—	—	0.00

the organisms may be possible, altho no data are available to substantiate such a statement other than that of a parallel case in which $NH_4H_2PO_4$ was used as the only source of nitrogen (see p. 36) in which very little autolysis appears to have taken place.

From these observations it is evident that accurate results can be obtained without freeing the medium of organisms before making the various analyses when studying nitrogen changes, and that the organisms in such concentrations as usually are found in nutrient media will not materially affect the results. It seems that our biochemical

methods are not sufficiently delicate to detect quantitatively the traces of nitrogenous substances found in bacterial cells when the organisms are not concentrated beyond the number normally present in growing cultures.

Larger amounts of culture suspensions may, however, give demonstrable results. Cultures of *M. varians* and *M. aurantiacus* were grown in large flasks of opsine broth for eight weeks, centrifuged, and the organisms suspended in small amounts of sterile physiological saline in such concentrations as to be nearly viscous. In these cases a definite biuret could be demonstrated and a Van Slyke determination showed appreciable amounts of amino nitrogen present. The cleared

TABLE 7.—EFFECT OF SUSPENDED ORGANISMS IN THE MEDIUM BEING TESTED UPON THE RESULTS WHEN DIFCO PEPTONE IS USED AS THE SOURCE OF NITROGEN.

No. of cult're	Name	Time of incu.	Amino-acid nitrogen (Van Slyke)		Amino-acid nitrogen (formol)		Biuret	
			Media	Organisms	Media	Organisms	Media	Organisms
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*		
270	*M. aurantiacus*..	1	0.4626	0.2280	0.280	0.175	1.20	0.00
270	*M. aurantiacus*..	3	0.6771	0.2805	0.490	0.210	1.00	0.00
270	*M. aurantiacus*..	6	0.5535	0.2767	0.420	0.070	1.00	0.00
270	*M. aurantiacus*..	9	0.7812	0.3348	0.490	0.140	1.00	0.00
270	*M. aurantiacus*..	14	0.8953	0.2797	0.490	0.070	1.00	0.00
270	*M. aurantiacus*..	30	0.7936	0.2870	0.350	0.070	1.00	—
	Uninoculated...	—	0.5134	—	0.210	—	1.20	—
349	*M. cinnebareus*.	1	0.3492	0.2280	0.210	0.190	1.20	0.00
349	*M. cinnebareus*.	3	0.6171	0.2244	0.420	0.210	1.00	0.00
349	*M. cinnebareus*.	6	0.6652	0.2767	0.350	0.070	1.00	0.00
349	*M. cinnebareus*.	9	1.0044	0.2790	0.420	0.070	1.00	0.00
349	*M. cinnebareus*.	14	1.0071	0.2797	0.560	0.140	1.00	0.00
349	*M. cinnebareus*.	30	0.8510	0.3444	0.280	0.140	0.90	—
	Uninoculated...	—	0.5134	—	0.210	—	1.20	—
80	*M. varians*.....	1	0.4626	0.2825	0.140	0.140	1.20	0.00
80	*M. varians*.....	3	0.6771	0.2805	0.560	0.175	1.00	0.00
80	*M. varians*.....	6	0.7195	0.2214	0.420	0.210	1.00	0.00
80	*M. varians*.....	9	0.6696	0.3348	0.630	0.140	1.00	0.00
80	*M. varians*.....	14	0.7380	0.2238	0.630	0.070	1.00	0.00
80	*M. varians*.....	30	0.9084	0.2870	0.630	0.140	1.00	0.00
	Uninoculated...	—	0.5134	—	0.210	—	1.20	—
386	*M. albus*.....	1	0.4626	0.2280	0.210	0.210	1.20	0.00
386	*M. albus*.....	3	0.6771	0.2244	0.420	0.210	1.00	0.00
386	*M. albus*.....	6	0.6652	0.2214	0.420	—	1.00	0.00
386	*M. albus*.....	9	0.8928	0.3348	—	0.140	1.00	0.00
386	*M. albus*.....	14	0.6785	0.3392	0.420	0.140	1.00	0.00
386	*M. albus*.....	30	0.9084	0.2870	0.420	0.140	1.00	0.00
	Uninoculated...	—	0.5134	—	0.210	—	1.20	—

TABLE 8.—EFFECT OF SUSPENDED ORGANISMS IN THE MEDIUM BEING TESTED UPON THE RESULTS WHEN OPSINE IS USED AS A SOURCE OF NITROGEN.

No. of culture	Name	Time of incu.	Amino-acid nitrogen (Van Slyke)		Amino-acid nitrogen (Formol)		Biuret	
			Media	Organisms	Media	Organisms	Media	Organisms
		Days	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*	*Mgs. per cc.*
270	*M. aurantiacus*	1	0.6804	0.3492	0.350	0.228	0.00	0.00
270	*M. aurantiacus*	3	0.6171	0.2805	0.490	0.210	0.00	0.00
270	*M. aurantiacus*	6	0.5520	0.2767	0.420	0.210	0.20	0.00
270	*M. aurantiacus*	9	0.5580	0.3348	0.420	0.775	0.20	0.00
270	*M. aurantiacus*	14	0.5595	0.3392	0.350	0.210	0.20	0.00
270	*M. aurantiacus*	35	0.3444	0.2870	0.420	0.140	0.20	0.00
	Uninoculated	—	0.2825	—	0.490	—	0.00	—
349	*M. cinnebareus*	1	0.6237	0.2845	0.420	0.210	0.00	0.00
349	*M. cinnebareus*	3	0.6171	0.3366	0.630	0.140	0.00	0.00
349	*M. cinnebareus*	6	0.5520	0.2767	0.490	0.140	0.20	0.00
349	*M. cinnebareus*	9	0.5022	0.2790	0.350	0.210	0.20	0.00
349	*M. cinnebareus*	14	0.5134	0.3392	0.420	0.210	0.20	0.00
349	*M. cinnebareus*	35	0.2870	0.2870	0.350	0.210	0.20	0.000
	Uninoculated	—	0.2825	—	0.490	—	0.00	—
80	*M. varians*	1	0.5193	0.2280	0.420	0.175	0.00	0.00
80	*M. varians*	3	0.5610	0.2805	0.560	0.210	0.00	0.00
80	*M. varians*	6	0.5520	0.3112	0.490	0.210	0.20	0.00
80	*M. varians*	9	0.5580	0.2790	0.280	0.175	0.20	0.00
80	*M. varians*	14	0.5134	0.2797	0.280	0.210	0.20	0.00
80	*M. varians*	35	0.5166	0.3444	0.210	0.210	0.20	0.00
	Uninoculated	—	0.2825	—	0.490	—	0.00	—
386	*M. albus*	1	0.6804	0.3492	0.560	0.175	0.00	0.00
386	*M. albus*	3	0.6771	0.2805	0.450	0.175	0.00	0.00
386	*M. albus*	6	0.4968	0.3112	0.630	0.210	0.20	0.00
386	*M. albus*	9	0.5022	0.2790	0.490	0.210	0.20	0.00
386	*M. albus*	14	0.5134	0.3392	0.420	0.210	0.20	0.00
386	*M. albus*	35	0.5470	0.4013	0.140	0.210	0.20	0.00
	Uninoculated	—	0.2825	—	0.490	—	0.00	—

opsine medium, however, even after this extended incubation, remained practically biuret-free.

ACTION OF MICROCOCCI UPON GELATIN

Levine and Carpenter (1923) have shown that liquefaction of gelatin or a decrease of its viscosity does not necessarily signify a hydrolysis of the gelatin and have further corroborated the conclusions of Berman and Rettger (1918) that the ability of an organism to liquefy gelatin does not indicate that such an organism is proteolytic.

In order to determine the actual nitrogen changes taking place when micrococci are grown in gelatin the following medium was made

TABLE 9.—THE ACTION OF THE MICROCOCCI UPON GELATIN.

No. of culture	Name	Time of incubation	Amino-acid nitrogen		Biuret	Liquefaction
			Van Slyke	Formol titration		
		Days	*Mgs. per cc.*	*Mgs. per cc.*		
385	*M. aureus*	1	1.0311	1.400	1.8	+
385	*M. aureus*	3	1.1280	1.820	1.8	+
385	*M. aureus*	6	1.2972	2.450	1.8	+
385	*M. aureus*	9	1.3846	2.520	1.8	+
385	*M. aureus*	14	1.4585	2.380	1.6	+
385	*M. aureus*	30	1.5827	2.380	1.6	+
	Uninoculated	—	0.8022	1.400	1.8	—
238	*M. albus*	1	0.8022	1.960	1.8	+
238	*M. albus*	3	1.1844	1.960	1.8	+
238	*M. albus*	6	1.2690	2.380	1.8	+
238	*M. albus*	9	1.2436	2.380	1.8	+
238	*M. albus*	14	1.5705	2.520	1.6	+
238	*M. albus*	30	1.5827	2.450	1.6	+
	Uninoculated	—	0.8022	1.400	1.8	—
196	*M. flavus*	1	1.0311	1.820	1.8	+
196	*M. flavus*	3	1.0816	2.100	1.8	+
196	*M. flavus*	6	1.1280	2.240	1.8	+
196	*M. flavus*	9	1.3000	2.380	1.8	+
196	*M. flavus*	14	1.0900	2.520	1.8	+
196	*M. flavus*	30	1.4135	2.520	1.6	+
	Uninoculated	—	0.8022	1.400	1.8	—
349	*M. cinnebareus*	1	1.0597	2.1700	1.8	+
349	*M. cinnebareus*	3	1.0534	2.240	1.8	+
349	*M. cinnebareus*	6	1.1280	2.240	1.8	+
349	*M. cinnebareus*	9	1.4130	2.310	1.8	+
349	*M. cinnebareus*	14	1.1740	2.520	1.6	+
349	*M. cinnebareus*	30	1.4135	2.660	1.6	+
	Uninoculated	—	0.8022	1.400	1.8	—
62	*M. conglomeratus*	1	1.0597	1.4000	1.8	+
62	*M. conglomeratus*	3	1.0534	2.2400	1.8	+
62	*M. conglomeratus*	6	1.1280	2.240	1.8	+
62	*M. conglomeratus*	9	1.2718	2.380	1.8	+
62	*M. conglomeratus*	14	1.1740	2.450	1.6	+
62	*M. conglomeratus*	30	1.4417	2.520	1.6	+
	Uninoculated	—	0.8022	1.400	1.8	—
88	*M. ureae*	1	1.1460	1.6800	1.8	+
88	*M. ureae*	3	1.1562	2.170	1.8	+
88	*M. ureae*	6	1.2408	2.310	1.8	+
88	*M. ureae*	9	1.1590	2.450	1.6	+
88	*M. ureae*	14	1.0620	2.310	1.6	+
88	*M. ureae*	30	1.3000	2.660	1.6	+
	Uninoculated	—	0.8022	1.400	1.8	—
213	*M. roseus*	1	1.1169	2.520	1.8	—
213	*M. roseus*	3	1.1280	2.100	1.8	—
213	*M. roseus*	6	1.1562	2.170	1.8	—
213	*M. roseus*	9	1.1026	2.240	—	—
213	*M. roseus*	14	0.9230	2.170	1.6	—

TABLE 9.—THE ACTION OF THE MICROCOCCI UPON GELATIN.—*Concluded.*

No. of culture	Name	Time of incubation	Amino-acid nitrogen		Biuret	Liquefaction
			Van Slyke	Formol titration		
		Days	*Mgs. per cc.*	*Mgs. per cc.*		
213	*M. roseus*	30	0.2545	2.300	1.6	—
	Uninoculated	—	0.8022	1.400	1.8	—
111	*M. casei*	1	0.9739	1.680	1.8	+
111	*M. casei*	3	1.1844	2.240	1.8	+
111	*M. casei*	6	1.6426	2.380	1.8	+
111	*M. casei*	9	1.3846	2.520	1.6	+
111	*M. casei*	14	1.6465	2.380	1.6	+
111	*M. casei*	30	1.6391	2.590	1.6	+
	Uninoculated	—	0.8022	1.400	1.8	—
191	*M. epidermidis*	1	0.8595	1.470	1.8	—
191	*M. epidermidis*	3	0.8624	1.710	1.8	—
191	*M. epidermidis*	6	0.9224	1.680	1.8	—
191	*M. epidermidis*	9	0.8393	1.750	1.8	—
191	*M. epidermidis*	14	0.8135	1.680	1.8	—
191	*M. epidermidis*	30	0.5936	1.820	1.8	—
	Uninoculated	—	0.8022	1.400	1.8	—
167	*M. citreus*	1	1.0311	2.170	1.8	—
167	*M. citreus*	3	1.0816	2.240	1.8	+
167	*M. citreus*	6	1.1280	2.100	1.8	+
167	*M. citreus*	9	1.1872	2.380	1.8	+
167	*M. citreus*	14	0.9780	2.380	1.6	+
167	*M. citreus*	30	1.3004	2.660	1.6	+
	Uninoculated	—	0.8022	1.400	1.8	—
80	*M. varians*	1	0.8595	1.400	1.8	—
80	*M. varians*	3	0.8346	1.560	1.8	—
80	*M. varians*	6	0.7050	1.710	1.8	—
80	*M. varians*	9	0.7065	1.750	1.8	—
80	*M. varians*	14	0.7293	1.750	1.8	—
80	*M. varians*	30	0.6218	1.620	1.8	—
	Uninoculated	—	0.8022	1.400	1.8	—

and inoculated with the eleven strains of micrococci under investigation.

Gelatin (Difco) ..... 40 grams
H_2O (distilled) ..... 1.0 liter.
Reaction adjusted to pH 7.0

The inoculated gelatin was incubated at 30° C. and tested at intervals similar to those employed for peptone.

It is interesting to note (Table 9) that seven of the eleven strains are liquefiers and, as observed in the case of peptone (Table 3), none of these cultures are proteolytic. The growth in the proteose-plus-glucose medium would not necessitate the splitting of the proteose molecule, but as stated above the organisms could secure sufficient nitrogen for growth by attacking the loosely joined amino groups.

M. aureus, *M. albus*, *M. flavus*, *M. cinnebareus*, *M. conglomeratus*, *M. ureae*, *M. casei*, and *M. citreus* are evidently types which decrease the viscosity to the point of liquefaction and also increase markedly the amount of demonstrable amino nitrogen. *M. varians* and *M. epidermidis* decrease slightly the amount of amino nitrogen without liquefaction, while *M. roseus* shows a marked decrease in amino nitrogen without liquefaction. This last group appears to be different from any of the three groups as determined by Levine and Carpenter, while the liquefying micrococci probably correspond to their third group which, according to definition, liquefy and completely hydrolyze the gelatin. None of the three liquefying strains had any apparent effect upon the biuret reaction, except for a slight decrease after two weeks' incubation in the case of *M. roseus*.

Many investigators working with this group have suggested that a constant and important diagnostic feature distinguishing between *M.aureus* and *M.albus* is the fact that the latter is invariably a slower liquefier. It seems, however, that such is not the case from observations made in this investigation. The rate of liquefaction and the time of the initial appearance of liquefaction may vary, due probably to the vigor of the culture as strains which have been transferred rapidly several times will liquefy more rapidly than old stock strains which have been carried without transferring for some time. Freshly isolated strains do not seem to have a greater liquefying power than old strains, providing the latter are invigorated before testing by a few transfers.

ACTION OF MICROCOCCI UPON $NH_4H_2PO_4$ WHEN FURNISHED AS THE ONLY SOURCE OF NITROGEN

In a previous part of this investigation (Technical Bulletin No. 100), it has been reported that the micrococci differ markedly in their ability to utilize ammonium salts as the only source of nitrogen when supplied in a solid medium, and it has been suggested that the utilization or non-utilization of this nitrogenous salt may furnish a basis for separating the truly parasitic from the saprophytic species of micrococci. When further investigated with the use of synthetic liquid media, however, this relationship is not found to furnish as sharp a division point as had previously been expected.

Cultures of the more strictly parasitic types, (Table 14) like *M. aureus*, *M. albus*, and *M. citreus*, in all cases failed to develop on such a synthetic medium, while *M. roseus*, *M. ureae*, *M. casei*, *M. varians*,

and *M. conglomeratus*, which may be classed as saprophytes, grew in practically all instances. However, *M. epidermidis* and *M. flavus* represent a group of micrococci which appear more or less variable in their relationships and which could be acclimated to produce a comparatively abundant growth with such an ammonium salt as the only source of nitrogen. Among the group which could be classed as "ammonium positive" *M. conglomeratus* and *M. ureae* appeared to grow when washed suspensions of organisms were used as an inoculum, while *M. roseus* generally produced little or no growth if cultures were washed before being used as an inoculum. Evidently, there is elaborated in these particular strains an ectoenzyme capable of attacking the ammonium salt. The probability of the presence of such an

TABLE 10.—THE ACTION OF THE MICROCOCCI UPON $NH_4H_2PO_4$ WHEN FURNISHED AS THE ONLY SOURCE OF NITROGEN.

No. of culture	Name	Time of incubation	Amino-acid nitrogen		Turbidity	Biuret
			Van Slyke	Formol titration		
		Days	*Mgs. per cc.*	*Mgs. per cc.*		
62	*M. conglomeratus*	1	0.1124	0.085	0.25	0.00
62	*M. conglomeratus*	3	0.1124	0.085	0.50	0.20
62	*M. conglomeratus*	6	0.1665	0.110	0.50	0.20
62	*M. conglomeratus*	9	0.1942	0.110	1.00	0.20
62	*M. conglomeratus*	14	0.1957	0.140	1.00	0.20
62	*M. conglomeratus*	30	0.2520	0.140	1.00	0.20
	Uninoculated	—	0.1124*	0.190	—	0.00
88	*M. ureae*	1	0.1124	0.110	0.25	0.00
88	*M. ureae*	3	0.1406	0.085	0.50	0.20
88	*M. ureae*	6	0.1942	0.110	1.00	0.20
88	*M. ureae*	9	0.1665	0.160	1.00	0.20
88	*M. ureae*	14	0.2517	0.160	1.00	0.20
88	*M. ureae*	30	0.2797	0.160	1.00	0.30
	Uninoculated	—	0.1124*	0.190	—	0.00
111	*M. casei*	1	0.1124	0.085	0.25	0.00
111	*M. casei*	3	0.1687	0.085	0.50	0.20
111	*M. casei*	6	0.1665	0.110	1.00	0.20
111	*M. casei*	9	0.1942	0.190	1.00	0.20
111	*M. casei*	14	0.1957	0.190	1.00	0.20
111	*M. casei*	30	0.3075	0.190	1.00	0.20
	Uninoculated	—	0.1124*	0.190	—	0.00
80	*M. varians*	1	0.1124	0.110	0.25	0.00
80	*M. varians*	3	0.1124	0.140	0.50	0.20
80	*M. varians*	6	0.1110	0.140	1.00	0.20
80	*M. varians*	9	0.1387	0.110	1.00	0.20
80	*M. varians*	14	0.1678	0.140	1.00	0.20
80	*M. varians*	30	0.2520	0.220	1.00	0.40
	Uninoculated	—	0.1124*	0.190	—	0.00

*Includes the blank reading using only the reagents.

enzyme was further demonstrated by adding as an inoculum, *M. roseus* from ammonium phosphate cultures of these organisms without washing, with the result that a much heavier growth followed than if inoculated with a washed inoculum direct from a nutrient agar slant.

The failure of *M. aureus*, *M. albus*, and *M. cinnebareus* to develop appears to be a constant characteristic and in no instance with a long series of cultures has this particular character varied.

The action upon the ammonium phosphate of those organisms which utilize such a source of nitrogen is outlined in Table 10.

In all cases, after a period of incubation, there appeared to be a gradual increase in the amount of amino acid present, whether determined by the formol titration or the Van Slyke methods. This is to be expected when it is noted that a sharp rise in the biuret occurs. The increased biuret is more pronounced than can be indicated by the scale used. Altho the readings are similar to those obtained with the amino-acid extract of peptone (Table 5), the biuret on the ammonium phosphate medium appeared more quickly and was more pronounced. It was of the purple-bluish color characteristic of proteose linkage, while that secured from the amino acid extract was of the pink variety usually associated with peptones.

It is evident that there is a possibility that such an increase in biuret may be due to autolytic products thrown out into the medium. In order to secure data on this point the test organisms were grown in broth, washed several times, and saline suspensions added to both ammonium phosphate medium and to an equal amount of physiological saline. The two sets of tubes were incubated at an optimum temperature and tested on the first and third day of growth. When a test was made the medium was cleared of organisms by centrifuging and the supernatant fluid tested. It was thought that autolysis, but not growth, would take place in the saline, and that the autolytic products could be demonstrated in the medium; while the organism would grow in the ammonium phosphate medium and true growth products could be discerned in this case. If no autolysis was found in the saline, it could be assumed that no autolysis would be found in the synthetic medium.

It can be seen from Table 11 that no autolyses with demonstrable by-products took place in the saline. This is indicated by no increase in biuret or amino nitrogen after the organisms had been removed. On the other hand, in the ammonium phosphate medium,

an increase in amino acid reading and increased biuret signifies that either simple synthetic products are formed and thrown into the surrounding medium or complex nitrogenous substances are formed in the cell from the ammonia and later are broken down into soluble biuret-giving substances which appear as such in the surrounding medium.

TABLE 11.—TO DETERMINE IF THE DEVELOPMENT OF BIURET IN AN INORGANIC SYNTHETIC MEDIUM IS DUE TO BACTERIAL AUTOLYSIS.

No. of culture	Name	Time of incubation	Supernatant fluid from ammonium phosphate media inoculated with saline suspension of organisms, incubated, and centrifuged		Supernatant fluid from saline inoculated with saline suspension of organisms, incubated, and centrifuged	
			Amino-acid nitrogen (Van Slyke)	Biuret	Amino-acid nitrogen (Van Slyke)	Biuret
		Days	*Mgs. per cc.*		*Mgs. per cc.*	
88	*M. ureae*.....	1	0.3093	1.00	0.1124	0.00
88	*M. ureae*.....	3	0.3348	2.00	0.1116	0.00
191	*M. epidermidis*	1	0.2248	1.00	0.1124	0.00
191	*M. epidermidis.*	3	0.2511	2.00	0.1124	0.00
62	*M. congl'm'r't's*	1	0.1687	1.00	0.1124	0.00
62	*M. congl'm'r't's*	3	0.1953	2.00	0.1116	0.00
	Uninoculated.	—	0.1124	0.00	0.1124	0.00

From such results one may conclude that some species of micrococci have the ability to utilize certain ammonium salts and synthesize higher nitrogenous compounds. The latter are thrown off and are demonstrable in the surrounding medium.

MINIMUM AMOUNTS OF NITROGEN REQUIRED BY MICROCOCCI

It has been shown by Rettger and his co-workers that bacteria, altho utilizing large amounts of carbon for energy, need only small amounts of nitrogen in building the cell. The minimum amounts of nitrogen required by the micrococci are shown in Tables 12 and 14.

The minimum amount of peptone (Table 12) required for growth is 0.001 per cent (by weight), while *M. ureae* can grow with a minimum of 0.0001 per cent of $NH_4H_2PO_4$. That organisms require more peptone than ammonium phosphate for growth is probably due to the fact that a greater amount of available nitrogen is offered in the same percentage of the ammonium salt than in the peptone.

In studying the minimum nitrogen requirements an interesting correlation can be found in Table 12. With the exception of *M. roseus*, the cultures failing to grow on ammonium phosphate require a greater amount of peptone to initiate growth than those which grow with ammonium phosphate as the only source of nitrogen. No apparent explanation can be found for this apparent and striking correlation.

NON-PROTEIN SOURCES OF NITROGEN FOR THE MICROCOCCI

Altho many of the micrococci can develop in media containing ammonium salts as the only source of nitrogen, none of the strains studied were able to grow when pure amino acids were furnished. This observation bears out the conclusions made by many other observers that, altho requiring amino nitrogen and even growing profusely in the presence of a biuret-free amino acid solution formed by digestion, pure amino acids will not promote growth. Some unknown factor must be present, such as vitamines, growth-accessory substances, or some undetermined substance. (See Table 13.)

Various ammonium salts of organic acids appear to furnish nitrogen as available as that of the phosphate. However, the ability to break the uric acid ring to secure nitrogen as demonstrated by offering uric acid, allantoin, etc., seems to be nearly lacking. In some instances small amounts of growth were observed.

The ability to attack urea appears to be associated with only one species of the genus *Micrococcus*, i. e., *M. ureae*. This character is probably the chief diagnostic feature of this particular organism and Cohn concluded that $(NH_4)_2CO_3$ was the product of this utilization.

DISCUSSION AND CONCLUSIONS

Among bacteria a great variation is found in regard to the form in which nitrogen is readily available. There are, on one hand, the nitrifiers which have the power of assimilating free nitrogen, and, on the other hand, such highly parasitic forms as the gonococci, etc., which require ammonia or amino nitrogen but probably in complex linkages of various complex animal substances as found in blood serum, ascitic fluids, etc. In all probability, all bacteria, other than the nitrifiers, utilize nitrogen as NH_2 or NH_3, but the requirements as to the source of such groups may vary. From the standpoint of nitrogen metabolism, the micrococci appear to be a group, having saprophytic tendencies and altho producing luxuriant growths on complex substances, they have retained the power to utilize more simple forms of nitrogen.

TABLE 12.—MINIMUM AMOUNT OF PEPTONE REQUIRED FOR GROWTH BY THE MICROCOCCI.

No. of culture	Name	Amount of peptone (per cent)								
		0.50	0.25	0.125	0.0625	0.031	0.0156	0.007	0.003	0.001	0.0009
385	*M. aureus*	+++	++	++	+	—	—	—	—	—	—
238	*M. albus*	+++	+++	++	—	—	+	—	—	—	—
349	*M. cinnebareus*	+++	+++	++	—	+	+	—	—	—	—
285	*M. aureus*	+++	+++	++	+	—	—	—	—	—	—
196	*M. flavus*	+++	++	—	+	+	—	—	—	—	—
62	*M. conglomeratus*	+++	+++	+++	++	++	++	+	+	+	—
88	*M. ureae*	+++	++	+++	++	++	++	+	+	+	—
213	*M. roseus*	+++	+++	++	++	+	—	—	—	—	—

TABLE 13.—AVAILABILITY OF VARIOUS NON-PROTEIN FORMS OF NITROGEN FOR THE MICROCOCCI.

No. of culture	Name	Source of nitrogen																
		Urea	Ammonium phosphate	Ammonium citrate	Ammonium oxalate	Ammonium tartrate	Uric acid	Hypoxanthine	Guanine	Tyrosine	Tryptophane	Histidine	Arginine	Asparagin	Leucine	Lysin	Glycollic acid	Valine
385	*M. aureus*	—	—	—	—	—	+	—	±	—	—	—	—	—	—	—	—	—
238	*M. albus*	—	—	—	—	—	+	—	±	—	—	—	—	—	—	—	—	—
196	*M. flavus*	—	±	—	+	+	+	±	+	—	—	—	—	—	—	—	—	—
349	*M. cinnebareus*	—	—	±	±	—	—	—	—	—	—	—	—	—	—	—	—	—
213	*M. roseus*	—	++	++	++	++	+	+	+	—	—	—	—	—	—	—	—	—
62	*M. conglom'r't's*	—	++	++	++	++	+	+	+	—	—	—	—	—	—	—	—	—
191	*M. epidermidis*	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
167	*M. citreus*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
80	*M. varians*	—	++	+	+	++	—	—	—	—	—	—	—	—	—	—	—	—
88	*M. ureae*	+++	++	++	++	++	—	—	—	—	—	—	—	—	—	—	—	—
111	*M. casei*	—	++	++	++	++	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 14.—MINIMUM AMOUNT OF $NH_4H_2PO_4$ REQUIRED FOR GROWTH BY THE MICROCOCCI.

No. of culture	Name	Amount of ammonium phosphate (per cent)		
		.01	.001	.0001
80	*M. varians*	+	—	—
88	*M. ureae*	+++	++	+++
191	*M. epidermidis*	++	—	—
62	*M. conglomeratus*	+	—	—

However, they have become either too specialized to use chemically pure amino acids or have lost the power to elaborate enzymes which would enable them to utilize such forms of nitrogen. In this respect, they differ from the more saprophytic forms of the colon-typhoid group which, in many cases, produce abundant growth in the presence of pure amino acids as an only source of nitrogen. In the presence of biuret-free digestion products, however, growth may signify that certain unknown growth-accessory substances may be present to initiate development, which once started can be carried on when pure amino acids only are furnished.

In the light of the above studies of the nitrogen requirements, the division of the micrococcus group into two genera does not seem warranted. The so-called *Staphylococcus* of which *Staphylococcus aureus* has been used as the type species has been placed in the same general group with the *Streptococcus* by various modern systematists, while the supposedly saprophytic *Micrococcus* group has been associated with the genus *Sarcina*. From the standpoint of availability of various nitrogen sources the organisms of the genera *Staphylococcus* and *Streptococcus* have little in common. The latter comprise a group which grow very scantily in the presence of peptones and polypeptids, requiring for maximum growth amino groups which are furnished directly from complex protein linkage. They are similar to the pneumococci, gonococci, etc., in this respect, while the staphylococci utilize nitrogen from a wide range of sources.

As can be seen from the above results which were carried out with representative cultures of the entire *Staphylococcus-Micrococcus* group, there appears to be little or no difference in the nitrogen metabolism of the various species. Other than the few differences already noted, these organisms appear to be closely associated and to fall into a single natural group.

The *Micrococcus* group is not proteolytic. When offered pure proteose, growth failed to appear. However, a large majority liquefy

gelatin in the absence of a fermentable carbohydrate. This observation offers further evidence that gelatin liquefaction does not imply proteolysis, but that the decrease in the viscosity of the gelatin may be due to an enzyme or to the destruction of closely linked amino groups or even to a splitting of the gelatin molecule. Even if an organism has this power, it does not follow that such an organism can attack proteoses.

The ability of this group to synthesize certain unknown substances offers an interesting observation. Biuret-giving substances were found to be present in very minute traces when the test organisms were grown in a biuret-free extract of commercial peptone, or when grown in the presence of opsine. However, in such instances, the biuret-giving substances did not appear until after several days of incubation. If, on the other hand, ammonia was furnished as the only source of nitrogen a distinct biuret will appear within a relatively short time. Evidently, the processes involved in assimilating NH_2 and NH_3 groups are materially different. The ammonia is either assimilated faster with the formation of biuret-giving products, or a smaller amount of the protein being synthesized from NH_3 is available than the corresponding biuret-giving substances formed from NH_2. If such is the case, the extra unavailable protein is thrown into the surrounding medium. On the other hand, when amino nitrogen is furnished, a larger portion of the synthesized protein may be available. It is evident, however, that the assimilation and utilization of NH_3 and NH_2 forms of nitrogen involves an entirely different series of chemical processes.

The association of the micrococcus group with various foods, particularly cheese, has led many workers in this field to connect closely the micrococci with ripening changes in cheese, more especially cheddar cheese. It is evident from these results that this group would be unable to exert a sufficient proteolytic action on the casein present to account for the complete ripening process. However, as can be seen from the action of certain strains, by the so-called acid-proteolytic properties some types may be connected with the little-known phenomena of flavor production.

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